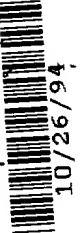


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APPLICATION FOR UNITED STATES PATENT

Inventor(s): BJORCK, Lars & SJOBRING, Ulf

Invention: PROTEIN L AND HYBRID PROTEINS THEREOF

Cushman Darby & Cushman, U.L.P.  
1100 New York Avenue, N.W.  
Ninth Floor, East Tower  
Washington, D.C. 20005-3918  
Attorneys  
Telephone: (202) 861-3000

SPECIFICATION

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Protein L and Hybrid Proteins Thereof

5 The present invention relates to sequences of protein L which bind to light chains of immunoglobulins. The invention also relates to hybrid proteins of protein L having the ability to bind to light chains of all Ig and also to bind to light and heavy chains of immunoglobulin G, DNA-sequences which code for the proteins vectors that contain such DNA-sequences, host cells transformed by the vectors, methods for preparing the proteins, reagent apparatus for separating and identifying immunoglobulins, compositions and pharmaceutical compositions which contain the proteins.

10 The invention relates in particular to the DNA-sequence and to the amino acid sequence of the light-chain forming domains of protein L.

15 Proteins which bind to the constant domains (of high affinity) of the immunoglobulins (Ig) are known. Thus, protein A (from Staphylococcus aureus) (Forsgren, A. and Sjöquist, J. (1966) Protein A from Staphylococcus aureus. I. Pseudo-immune reaction with human gamma-globulin. J. Immunol. 97: 822-827) binds to IgG from various mammal species. The binding of protein A to IgG is mediated essentially via surfaces in the Fc-fragment of the heavy chain of the IgG-molecule, although a certain bond is also effected with surfaces in the Fab-fragment of the IgG. Protein A lacks the ability of binding to human IgG3 and neither will it bind to IgG from several other animal species, such as important laboratory animals, for instance rats and goats, which limits the use of protein A.

30 Protein G (Björck, L. and Kronvall, G. (1984) Purification and some properties of streptococcal protein G, a

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novel IgG-binding reagent. J. Immunol. 133: 969-974;  
Reis, K., Ayoub, E. and Boyle, M. (1984) Streptococcal  
Fc receptors. I. Isolation and partial characterization  
of the receptor from a group C streptococcus. J.

5 Immunol. 132: 3091-3097) binds to heavy chains in human  
IgG and to all four of its subclasses and also to IgG  
from most mammals, including rats and goats.

Protein H (Åkesson, P., Cooney, J., Kishimoto, F. and  
10 Björck, L. (1990) Protein H - a novel IgG binding bacte-  
rial protein. Molec. Immun. 27: 523-531) binds to the  
Fc-fragment in IgG from human beings, monkeys and rab-  
bits. However, the bond is weaker than in the case of  
protein G and A, which may be beneficial when wishing to  
15 break the bond with a weak agent, for instance when  
purifying proteins which are readily denatured with the  
aid of antibodies.

Protein M (Applicant's Patent Application PCT/SE  
20 91100447) binds to the Fc-fragment in IgG from humans,  
monkeys, rabbits, goats, mice and pigs.

Protein L (Björck, L. (1988) Protein L, a novel bacteri-  
al cell wall protein with affinity to Ig L chains. J.  
25 Immunol. 140: 1194-1197), which binds to the light  
chains in immunoglobulins from all of the classes G, A,  
M, D and E is known (USP 4,876,194). The amino acid se-  
quence and the binding domains of this protein, however,  
have hitherto been unknown.

30 The aforesaid proteins can be used in the analysis,  
purification and preparation of antibodies and for  
diagnostic and biological research.

35 The elimination of immunoglobulins, with the aid of

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plasmapheresis, can have a favourable effect on some autoimmune diseases. A broadly binding protein would be an advantage when wishing to eliminate all classes of antibodies in this context.

5 It has long been known that infectious conditions can be prevented or cured with the introduction of an immune serum, i.e. a serum which is rich in antibodies against the organism concerned or its potentially harmful product. Examples hereof are epidemic jaundice, tetanus, 10 diphtheria, rabies and generalized shingles. Antibodies against a toxic product may also be effective in the case of non-infectious occasioned conditions. Serum produced in animals against different snake venoms is 15 the most common application in this respect. However, the administration of sera or antibody preparations is not totally without risk. Serious immunological reactions can occur in some cases. Singular cases of the transmission of contagious diseases, such as HIV and 20 hepatitis through the agency of these products have also been described. In order to avoid these secondary effects, it has been desirable to produce therapeutic antibodies in test tubes. A large number of novel techniques for the preparation of antibodies in test tubes 25 have been proposed in recent years. Examples of such techniques are hybridoma techniques, synthesis of chima-antibodies and the preparation of antibodies in bacteria. These techniques also enable antibodies to be specially designed which can further widen the use of 30 such molecules as therapeutics, for instance in the case of certain tumour-diseases. In the case of some of these novel methods, however, the product totally lacks the Fc-fragment to which all of the described IgG-binding proteins, with the exception of protein L, bind. There 35 is consequently a need of a process for purifying anti-

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bodies for therapeutic use, wherein proteins which have a broad binding activity/specificity, can be of value.

5 It has long been possible to utilize the antibody reaction with its high grade specificity for diagnosing past or, in some cases, ongoing infections with different parasites. This indirect method of indicating infectious agents is called serology and, in many cases, may be the only diagnostic alternative. In certain cases, it can  
10 also be of interest to exhibit specific IgE- or IgA-antibodies. When diagnosing with the aid of serology, the antigen is most often fastened to a solid phase, whereafter serum taken from the patient is incubated with the antigen. Antibodies that have been bound from  
15 the patient can then be detected in different ways, often with the aid of a secondary antibody (for instance, an antibody which is directed against the light chains of human antibodies) to which an identifiable label has been attached, such as alkaline phosphatase,  
20 biotin, radioactive isotopes, fluorescein, etc. In this context, a protein having a broad Ig binding capacity can be used as an alternative to secondary antibodies.

25 There are a number of non-therapeutic and non-diagnostic reasons for the necessity to bind antibodies. Antibodies are often used in research, both for detection and for purifying the antigen against which they are directed. All techniques which facilitate the purification of antibodies and, in particular, techniques which enable  
30 different classes to be purified, are of interest in this context.

Consequently, there is a serious need of a protein which has a broad binding activity/specificity and which binds  
35 to several different classes of immunoglobulins from different animal species. At present, there is no known

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protein which will bind to all immunoglobulin classes. The earlier known proteins A, G, H and M bind only to heavy chains in IgG. The known protein L (Björck et al, 1988) binds to the light  $\kappa$ -chains and  $\gamma$ -chains in immunoglobulins of all classes, although the bonds are much weaker on the  $\kappa$ -chains. Applicant has charted protein L, has determined the amino acid sequence for protein L, has identified the light-chain binding domains on protein L, and has used these to produce hybrid proteins which possess the IgG-Fc-binding domains of protein G. The Applicant is able to show through protein LG that a protein of broader binding activity/specificity can be produced thereby. The aforesaid proteins A, G, H and M bind to the same surfaces, or to very closely lying surfaces on IgG-Fc. The protein L which binds to light chains can thus be combined with any other functionally similar protein which binds to the Fc-fragment of heavy chains. A similar broadening of the Ig-binding activity is achieved with all alternatives.

Thus, the present invention relates to the sequence of protein L which binds to light chains in Ig and has the amino acid sequence disclosed in Figure 1, and variants, subfragments, multiples or mixtures of the domains B1-E5 having the same binding properties. The invention also relates to a DNA-sequence which codes for such protein sequences, for instance the DNA-sequence in Figure 1.

The invention is concerned with a hybrid protein which is characterized by comprising domains which bind to the light  $\kappa$ -chains and  $\lambda$ -chains in immunoglobulins of all classes, and also comprises domains which bind to heavy chains in immunoglobulin G, wherein those domains which bind to the light chains are chosen from among the B1-, B2-, B3-, B4- and B5-domains in protein L (see Claim 1)

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and those domains which bind to heavy chains of immunoglobulins are chosen from the C1-, C2- and C3-domains in protein G; the A-, B- and C1-domains from protein H; the A-, B1-, B2- and S-domains in protein M1 or the E-, D-, A-, B- and C-domains in protein A (see Figure 6) and variants, subfragments, multiples or mixtures of these domains that have the same binding properties which bind to heavy chains of immunoglobulins.

- 10 By subfragment is meant a part-fragment of the given domains or fragments which include parts from the various domains having mutually the same binding properties. By variants is meant proteins or peptides in which the original amino acid sequence has been modified or
- 15 changed by insertion, addition, substitution, inversion or exclusion of one or more amino acids, although while retaining or improving the binding properties. The invention also relates to those proteins which contain several arrays (multiples) of the binding domains or
- 20 mixtures of the binding domains with retained binding properties. The invention also relates to mixtures of the various domains of amino acid sequences having mutually the same binding properties.
- 25 The invention relates in particular to a hybrid protein designated LG, and is characterized in that the hybrid protein includes the B-domains in protein L which bind to the light chains in immunoglobulins, and the C1-domains and C2-domains in protein G which bind to heavy
- 30 chains and have the amino acid sequence disclosed in Figure 3. The invention also relates to variants, subfragments, multiples or mixtures of these domains.

35 Protein LG is a hybrid protein having a molecular weight of about 50 kDa (432 amino acids) and comprising four domains, each of which binds to light chains in immuno-

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globulins, and two IgG-binding domains from protein G. The hybrid protein combines a broad IgG-binding activity, deriving from the high-grade binding ability of protein G to the Fc-fragment of the heavy chain on IgG with the ability of the protein L to bind to light chains of all classes of immunoglobulins. Thus, protein LG binds polyclonal human IgG, IgM, IgA, IgD and IgE. The affinity for human polyclonal IgG is  $2 \times 10^{10} \text{ M}^{-1}$ . All four human immunoglobulin classes are bound. Binding to human IgG is effected with both the  $\kappa$ - and the  $\lambda$ -chain. Both the Fc-fragment and the Fab-fragment of IgG are bound to the hybrid protein. The protein also binds human IgA-, IgD-, IgE- and IgM-antibodies. The bond is stronger to human immunoglobulins which carry  $\kappa$  than to those which carry the  $\lambda$ -isotope of light chains. IgG from most mammals will be bound by protein LG, thus also IgG from goats and cows, which do not bind to protein L. However, rabbit-IgG which binds relatively weakly to protein L will bind well to the fusion protein. IgM and IgA-antibodies from mice, rats and rabbits will be bound to the protein.

Protein LG is highly soluble. It is able to withstand heat and will retain its binding properties even at high temperatures. The binding properties also remain in a broad pH-range of 3-10. The protein withstands detergent and binds marked or labelled proteins subsequent to separation in SDS-PAGE and transference to membranes with elektroblotting. The protein can be immobilized on a solid phase (nitrocellulose, Immobilon®, polyacrylamide, plastic, metal and paper) without losing its binding capacity. The binding properties are not influenced by marking with radioactive substances, biotin or alkaline phosphatase. (The binding abilities of the protein LG are disclosed in Example 3).

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The protein comprises 432 amino acids and has a molecular weight of 50 kDa deriving therefrom. The sequence is constructed of an ala sequence of the three last amino acids in the A-domain of the protein L (val-glu-asn), this ala sequence being unrelated to the two proteins, whereafter the four mutually high-grade homologous B-domains from protein L follow. The first of the B-domains is comprised of 76 amino acids, and the remaining domains are each comprised of 72 amino acids. The first nine amino acids from the fifth B-domain are included and followed by two non-related amino acids (pro-met). The protein G-sequences then follow. The last amino acid in the so-called S-domain from protein G is followed by an IgG-binding domain from protein G (C1; 55 amino acids), the intermediate D-region (15 amino acids) and the second IgG-binding C-domain (C2; 55 amino acids). The last amino acid is a methionine, which occurs in natural protein G as the first amino acid in the so-called W-region.

The invention also relates to DNA-sequences which code for the aforesaid proteins.

The gene which codes for the IgG-binding amino acid sequences can be isolated from the chromosomal DNA from Staphylococcus aureus based on the information on the DNA-sequence for protein A (S. Löfdahl, B. Guss, M. Uhlen, L. Philipsson and M. Lindberg. 1983. Gene for staphylococcal protein A. Proc. Natl. Acad. Sci. USA. 80: 697-701) and Figure 6, or from G-streptococcus, preferably strain G 148 or C-streptococcus, preferably strain Streptococcus equisimilis C 40, based on the information on protein G (B. Guss, M. Eliasson, A. Olsson, M. Uhlen, A.-K. Frej, H. Jörvall, I. Flock and M. Lindberg. 1986. Structure of the IgG-binding

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regions c streptococcal protein G. EMBO. J. 5: 1567-1575) and Figure 6, or from group A-streptococcus, e.g. S. pyogenes (type M1) based on the information on the DNA-sequence for protein H (H. Gomi, T. Hozumi, S. Hattori, C. Tagawa, F. Kishimoto and L. Björck. 1990. The gene sequence and some properties of protein H - a novel IgG binding protein J. Immunol. 144: 4046-4052) and Figure 6, or from the chromosomal DNA in group A-streptococcus type M1 based on the information on the DNA-sequence for protein M (Applicant's Patent Application, PCT/SE 91100447) and Figures 6 and 7. The gene which codes for the protein that binds to light chains can be isolated from the chromosomal DNA from Pepto-coccus magnus 312 based on the information on the DNA-sequence for protein L in Claim 2.

By using the chromosomal DNA't obtained from the aforesaid bacteria as a template, a DNA-fragment defined with the aid of two synthetic oligonucleotides can then be specifically amplified with the aid of PCR (Polymerase Chain Reaction). This method also enables recognition sites to be incorporated for restriction enzymes in the ends of the amplified fragments (PCR technology, Ed: PCR Technology. Principles and Applications for DNA Amplification. Ed. Henry Erlich. Stockton Press, New York, 1989). The choice of recognition sequences can be adapted in accordance with the vector chosen to express the fragment or the DNA-fragment or other DNA-fragments with which the amplified fragment is intended to be combined. The amplified fragment is then cleaved with the restriction enzyme or enzymes concerned and is combined with the fragment/the other fragments concerned and the fragments are then cloned together in the chosen vector (in this case, the expression vector) (Sambrook, J.E. Fritsch and T. Maniatis, 1989, Molecular cloning: A laboratory manual, 2nd Ed. Cold Spring Harbor Laborato-

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- ries, Cold Spring Harbor, New York, USA). The plasmid vector pHD313 can be used (Dalbøge, H.E. Bech Jensen, H. Töttrup, A. Grubb, M. Abrahamsen, I. Olafsson and S. Carlsen, 1989. High-level expression of active human cystatin C in *Escherichia coli*. *Gene*, 79: 325-332), alternatively one of the vectors in the so-called PET-series (PET 20, 21, 22, 23) retailed by Novagen (Madison, Wisconsin, USA).
- 10 The hybrid proteins are then incorporated in an appropriate host, preferably *E. coli*. The invention also relates to such hosts as those in which the hybrid proteins are incorporated.
- 15 Those clones which produce the desired proteins can be selected from the resultant transformants with the aid of a known method (Fahnestock et al., *J. Bacteriol.* 167, 870 (1986).
- 20 When the proteins that can bind to the light chains in the immunoglobulins and to the heavy chains in IgG have been purified from the resultant positive clones with the aid of conventional methods, the binding specificities of the proteins are determined for selection of those clones which produce a protein that will bind to the light chains in immunoglobulins and to the heavy chains in IgG.
- 25
- Subsequent to having isolated plasmid DNA't in said clone with conventional methods, the DNA-sequence in the inserted material is determined with known methods (Sanger et al., *Proc. Natl. Acad. Sci. USA* 74, 5463 (1977).
- 30
- 35 The invention also relates to DNA-sequences which hybridize with said identified DNA-sequences under conven-

tional conditions and which code for a protein that possesses the desired binding properties. Strict hybridizing conditions are preferred.

5 Expression of the genes can be effected with expression vectors which have the requisite expression control regions, the structural gene being introduced after said regions. As illustrated in Figure 1 and Claim 2, the structural gene can be used for protein LG or other  
10 hybrid proteins with protein L.

With regard to expression vectors, different host-vector-systems have been developed, of which the most suitable host-vector-systems can be selected for expres-  
15 sion of the genes according to the present invention.

The present invention also relates to a method of producing the inventive hybrid proteins by cultivating a host cell which is transformed with an expression vector  
20 in which DNA't which codes for the proteins according to the invention is inserted.

This method includes the steps of

- 25 (1) inserting into a vector a DNA-fragment which codes for the hybrid proteins;
- (2) transforming the resultant vector into an appropriate host cell;
- 30 (3) cultivating the resultant, transformed cell for preparation of the desired hybrid protein; and
- (4) extracting the protein from the culture.

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In the first step, the DNA-fragment which codes for the hybrid protein is inserted in a vector which is suitable for the host that is to be used to express the hybrid protein. The gene can be inserted by cleaving the vector with an appropriate restriction enzyme, and then ligating the gene with the vector.

In the second step, the vector with the hybrid plasmid is inserted into host cells. The host cells may be Escherichia coli, Bacillus subtilis or Saccharomyces cerevisiae or other suitable cells. Transformation of the expressions hybrid vector into the host cell can be effected in a conventional manner and clones which have been transformed can then be selected.

In the third step, the obtained transformants are cultivated in an appropriate medium for preparation of the desired proteins by expression of the gene coded for the hybrid protein.

In the fourth step, the desired protein is extracted from the culture and then purified. This can be achieved with the aid of known methods. For instance, the cells can be lysed with the aid of known methods, by treating the cells with ultrasonic sound, enzymes or by mechanical degradation. The protein which is released from the cells or which excretes in the medium can be recovered and purified with the aid of conventional methods often applied within the biochemical field, such as ion-exchange chromatography, gel filtration, affinity chromatography with the use of immunoglobulins as ligands, hydrophobic chromatography or reverse-phase chromatography. These methods can be applied individually or in suitable combinations.

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As before mentioned, the inventive proteins may be used for binding, identifying or purifying immunoglobulins. They can also be bound to pharmaceuticals and used in formulations which have delayed release properties. To this end, the protein may be present in a reagent appli-  
5      ance for pharmaceutical composition in combination with appropriate reagents, additives or carriers.

The proteins can be handled in a freeze-dried state or in a PBS-solution (phosphate-buffered physiological salt solution) pH 7.2 with 0.02%  $\text{NaN}_3$ . It can also be used connected to a solid phase, such as carbohydrate-based phases, for instance CNBr-activated sepharose, agarose, plastic surfaces, polyacrylamide, nylon, paper, magnetic  
10      spheres, filter, films. The proteins may be marked with biotin, alkaline phosphatase, radioactive isotopes, fluorescein and other fluorescent substances, gold particles, ferritin, and substances which enable luminescence to be measured.

Other proteins may also be used as carriers. These carriers may be bound to or incorporated in the proteins, in accordance with the invention. For instance, it is conceivable to consider the whole of proteins A, G, H, M as carriers for inserted sequences of protein L  
15      which bind to light chains. In turn, these carriers can be bound to the aforesaid carriers.

The pharmaceutical additions that can be used are those which are normally used within this field, such as pharmaceutical qualities of mannitol, lactose, starch, magnesium stearate, sodium saccharate, talcum, cellulose, glucose, gelatine, saccharose, magnesium carbonate and similar extenders, such as lactose, dicalcium phosphate and the like; bursting substances, such as starch  
20      or derivatives thereof; lubricants such as magnesium

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stearate and the like; binders, such as starch, gum arabicum, polyvinylpyrrolidone, gelatine, cellulose and derivatives thereof, and the like.

5 The invention will now be described in more detail with reference to the accompany drawings, in which

Figure 1 illustrates the plasmid pHD389; the ribosomal binding sequence, the sequence for the signal peptide from ompA and recognition sequence for several restriction enzymes are shown;

Figure 2 illustrates the amino acid and nucleic acid sequence for protein LG;

15 Figure 3 is a schematic overall view of the production of protein L;

Figure 4 is a schematic overall view of the production of protein LG;

Figures 5a, 5b and 5c are schematic overall views of the production of the hybrid proteins LA, LM and LH respectively;

25 Figure 6 is a schematic inclusive illustration of protein A, G, H and M1. IgGFC-binding domains are for protein A: E, D, A, B and C; for protein G: C1, C2 and C3; for protein H: A and/or B; and for protein M1: A, B1, B2, B3 and S;

30 Figure 7 illustrates the amino acid and nucleic acid sequence for protein M1;

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Figure 8 illustrates Western Blot for protein G, L and LG with certain immunoglobulins and immunoglobulin fragments; and

5 Figure 9 illustrates Slot-Blot for protein L, G and LG with IgG, IgX and Ig Fc.

The amino acid and nucleic acid sequence of the light-chain binding domains of protein L is illustrated in  
10 Claims 1 and 2 respectively.

It will be observed that the drawings are not to scale.

Example 1

15

Cloning and expression of the IgG-light-chain-binding domains in Protein L

20

Construction of synthetic oligonucleotides (primers) for amplifying sequences coded for protein L, domain B1-B4

25

30

35

It has been found that a protein L peptide (expressed in E. coli) constructed of the sequence ala-val-glu-asn-domain B1 (from protein L) binds to the light chains of the immunoglobulins (W. Kastern, U. Sjöbring and L. Björck. 1992. Structure of peptostreptococcal protein L and identification of a repeated immunoglobulin light chain-binding domain. J. Biol. Chem. in-print). Since this simple protein L-domain has a relatively low affinity to Ig, ( $1 \times 10^7 \text{ M}^{-1}$ ), and since the naturally occurring protein L which is constructed of several mutually similar domains (B1-B5) has a high affinity to Ig ( $1 \times 10^{10} \text{ M}^{-1}$ ) four of these domains have been expressed together in the following way:

PL-N and PL-C1 are synthetic oligonucleotides (manufactured by the Biomolecular Unit at Lund University (Sweden) in accordance with Applicant's instructions) which have been used to amplify a clonable gene fragment which is amplified with PCR (Polymerase Chain Reaction) and which codes for four Ig-binding protein L domains (ala-val-glu-asn-B1-B2-B3-B4-lys-lys-val-asp-glu-lys-pro-glu-glu). Amino acids in the protein L-sequence are given for the primer which corresponds to the coded strand (PL-N):

PL-N: 5'-GCTCAGGCGGCGCCGGTAGAAAATAAAGAAGAAACACCAGAAAC-3'  
valgluasnlysglugluthrproglu

5'-end of this oligonucleotide is homologous with the coded strand in the protein L-gene (emphasized): those codons which code for the last three amino acids in the A-domain (val-glu-asn) are followed by the codons for the first six amino acids in the first of the Ig-binding domains in protein L (B1).

PL-C1: 5'-CAGCAGCAGGATTCTTATTATTCTTCTGGTTTTTCGTCAACTTTCTT-3'

This oligonucleotide is homologous with the opposing non-coding strand in the gene for protein L (the sequence corresponds to the first nine amino acids in domain B5).

DNA-fragments which have been amplified with the aid of PL-N contain the recognition sequence for the restriction enzyme HpaII (emphasized) immediately before the codon which is considered to code for the first amino acid (val) in the expressed protein L-fragment. The fragment which is cleaved with HpaII can be ligated with

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DNA (in this case, consisting of the used expression vector pHD389) which has been cleaved with the restriction enzyme NarI. The DNA-fragment that has been cleaved with HpaII and ligated with vector pHD389, which has been cleaved with NarI, will be translated in the correct reading frame. The construction results in translation of an additional amino acid (ala) immediately in front of the first amino acid in protein L.

DNA-fragments which have been amplified with the aid of PL-C1 will contain the recognition sequence for the restriction enzyme BamHI (overlined above the sequence) immediately after the sequence which codes for the last amino acid in the expressed protein L-fragment (glu). The vector pHD389 contains a unique recognition sequence for BamHI as part of its so-called multiple cloning sequence which follows the NarI recognition sequence. DNA-fragments which have been amplified with the aid of PL-C1 will include two so-called stop-codons (emphasized) which results in translation of the fragment inserted in the vector to cease.

The sequence which was considered to be amplified contains no internal recognition sequences for the restriction enzymes HpaII or BamHI.

#### Amplifying and cloning procedures

(PCR) (Polymerase Chain Reaction) was effected with a protocol described by Saiki, R.D. Gelfand, S. Stoffel, S. Schzrf, R. Higuchi, G. Horn, K. Mullis and H. Erlich, 1988; Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239: 487-49127; PCR was effected in a Hybaid Intelligent Heating-block (Teddington, UK): 100 µl of a reaction mixture contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM

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MgCl<sub>2</sub>, 100 µ/ml gelatine, 300 µM with respect to each of the deoxynucleotides (dATP, dCTP, dGTP, dTTP), (Pharmacia), 20 pmol of each of the oligonucleotides PL-N and PL-C1, 10 µl of a target (template) DNA-solution containing 0.1 mg/ml of chromosomal DNA from Pepto-  
5 streptococcus magnus, species 312. The mixture was covered with mineral oil (Sigma) and DNA't was denatured by heating to 98°C for 10 minutes. 2.5 units of AmpliTaq (Perkin Elmer Cetus, Norwalk, CT) were added and PCR was  
10 then carried out with 25 cycles consisting of a denaturing step at 94°C for 1 minute, followed by a hybridizing step at 56°C for 1 minute, and finally by an extension step at 72°C for 1 minute. Amplified DNA was analyzed by electrophoresis in agarose gel. The amplified DNA't was  
15 cleaved with the restriction enzymes HpaII (Promega), (8 units/µg amplified DNA) and BamHI (Promega), (10 units/µg amplified DNA) at 37°C. The thus amplified and subsequently cleaved DNA-product was isolated by electrophoresis in a 2% (weight by volume) agarose gel  
20 (NuSieve agarose, FMC Biproducs) in a TAE-buffer (40 Mm Tris, 20 Mm Na-acetate, 2 Mm EDTA, Ph 8.0). The resulting 930 base-pair large fragment was cut from the gel. The DNA concentration in this removed gel-piece was estimated to be 0.05 mg/ml. The agarose-piece containing  
25 the cleaved, amplified fragment was melted in a water bath at 65°C, whereafter the fragment was allowed to cool to 37°C. 10 µl (0.5 µg) of this DNA was transferred to a semimicrotube (Sarstedt) preheated to 37°C, whereafter 1 µl of the vector pHD389 was immediately added  
30 and cleaved with NarI (Promega) and BamHI, 1 µl 10xligas-buffer (Promega) and 1 µl T4 DNA-ligase (Promega; 1 unit/µl). The ligating reaction was then used to transform E. coli, strain LE392, which had been competent in accordance with the rubidium/calcium-chloride-method as described by Kushner (1978). Molecular biological  
35 standard methods have been used in the manipulation

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of DNA (Sambrook, J.E. Fritsch and T. Maniatis, 1989. Molecular cloning: A laboratory manual. 2nd Ed. Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, USA). The cleaving and ligating conditions recommended by the manufacturer of DNA-ligase and restriction enzymes have been followed in other respects.

#### Expression system

The vector pHD389 (see Figure 2) is a modified variant of the plasmid pHD313 (Dalbøge, H.E. Bech Jensen, H. Töttrup, A. Grubb, M. Abrahamson, I. Olafsson and S. Carlsen, 1989. High-level expression of active human cystatin C in *Escherichia coli*. Gene, 79: 325-332). The vector, which is replicated in *E. coli* (contains ori = origin of replication from plasmid pUC19) is constructed so that DNA-fragments which have been cloned into the cleaving site of *NarI* will be transcribed and translated downstream of and in the immediate vicinity of the signal peptide (21 amino acids), from envelope-protein *ompA* from *E. coli*. Translation will be initiated from the codon ATG which codes for the first amino acid (methionine) in the signal peptide. This construction permits the translated peptide to be transported to the periplasmic space in *E. coli*. This is advantageous, since it reduces the risk of degradation of the desired product of enzymes occurring intracellularly in *E. coli*. Moreover, it is easier to purify peptides which have been exported to the periplasmic space. Unique recognition sequences (multiple cloning sequences) for several other restriction enzymes, among them *ecoRI*, *SalI* and *BamHI* are found immediately after the *NarI* cleaving site. An optimized so-called Shine-Dalgarno-sequence (also called ribosomal binding site, RBS) is found seven nucleotides upstream from the ATG-codon in the signal sequence from *ompA*, this optimized sequence binding to a

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complementary sequence in 16S rRNA in the ribosomes and is responsible for the translation being initiated in the correct place. The transcription of such DNA as that which is co-transcribed with the signal sequence for ompA is controlled by the  $P_R$ -promotor from coliphage  $\lambda$ . The vector also contained the gene for cI857 from coliphage  $\lambda$  whose product down-regulates transcription from  $P_R$  (and whose product is expressed constitutively). This cI857-mediated down-regulation of transcription from  $P_R$  is heat-sensitive. The transcription regulated from this promoter is terminated with the aid of a so-called rho-independent transcription terminating sequence (forms a structure in DNA't which results in the DNA-dependent RNA-polymerase leaving the DNA-strand) which is placed in the vector immediately downstream of the multiple cloning sequence. The plasmid also carries the  $\beta$ -lactamase gene (from the plasmid pUC19) whose product permits ampicillin-selection of E. coli clones that have been transformed by the vector.

20

#### Selection of protein L-producing clones

The transformed bacteria are cultivated, or cultured, on culture plates with an LB-medium which also contained ampicillin in a concentration of 100  $\mu$ g/ml. Cultivation of the bacteria progressed overnight at 30°C, whereafter the bacteria were transferred to an incubator where they were cultivated for a further 4 hours at 42°C. The plates were kept in a refrigerator overnight. On the next day, the colonies were transferred to nitrocellulose filters. Filters and culture plates were marked so as to enable the transferred colonies to be readily identified on respective culture plates. The culture plates were again incubated overnight at 30°C, so that remaining rests of transferred bacteria colonies could again grow. The plates were then kept in a refrigerator.

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The bacteria in the colonies on the nitrocellulose-impressions were lysed by incubating the filter in 10% SDS for 10 minutes. Filters containing lysed bacteria were then rinsed with a blocking buffer which comprised  
5 PBS (pH 7.2) with 0.25% gelatine and 0.25% Tween-20 (four baths, 250 ml each at 37°C), whereafter the filter was incubated with radioactively marked (marked with <sup>125</sup>I in accordance with the chloramin-T-method) Ig- $\kappa$ -chains (20 ng/ml in PBS with 0.1% gelatine). The incubation took place at room temperature over a period of 3  
10 hours, whereafter non-bound radioactively marked protein was rinsed-off with PBS (pH 7.2) containing 0.5 M NaCl, 0.25% gelatine and 0.25% Tween-20 (four baths, 250 ml each at room temperature). All filters  
15 were exposed to X-ray film. Positive colonies were identified on the original culture plate. Clones which reacted with Ig- $\kappa$ -chains were selected and analyzed with respect to the size on the DNA-fragment introduced in the vector. One of these clones was selected for the  
20 production of protein L, pHDL. The DNA't introduced from this clone into plasmid pHD389 was sequenced. The DNA-sequence was found to be in full agreement with corresponding sequences (B1-B4 and 21 bases in B5) in the gene for protein L from Peptostreptococcus magnus,  
25 strain 312. The size and binding properties of the protein produced by clone pHDL was analyzed with the aid of SDS-PAGE (see Figure 8), dot-blot experiment (see Figure 9) and competitive binding experiments.

#### 30 Production of protein L

Several colonies from a culture plate with E. coli pHDL were used to inoculate a preculture (LB-medium with an addition of 100 mg/l ampicillin), which was cultured at  
35 28°C overnight. On the following morning, the preculture was transferred to a larger volume (100 times the volume

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of the preculture) of fresh LB-medium containing ampicillin (100 mg/l) and was cultured in shake-flasks (200 rpm), (or fermentors) at 28°C. The culture temperature was raised to 40°C (induction of transcription) when the absorbency value at 620 nm reached 0.5. Cultivation then continued for 4 hours (applied solely to cultivation in shake-flasks). Upon completion of the cultivation process, the bacteria were centrifuged down. The bacteria were then lysed with an osmotic shock method at 4°C (Dalbøge et al., 1989 supra). The lysate was adjusted to a pH = 7. Remaining bacteria rests were then centrifuged down, whereafter the supernatant was purified on IgG-sepharose in accordance with earlier described protocol for protein G and protein L (U. Sjöbring, L. Björck and W. Kastern. 1991. Streptococcal protein G: Gene structure and protein binding properties. J. Biol. Chem. 266: 399-405; W. Kastern, U. Sjöbring and L. Björck. 1992. Structure of peptostreptococcal protein L and identification of a repeated immunoglobulin light chain-binding domain. J. Biol. Chem. in-print.

The expression system gave about 20 mg/l of protein L when cultivation in shake-flasks. The culture was deposited at DSSM, Identification Reference DSSM E. coli LE392/pHDL.

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### Cloning and expression of protein LG

### Protein L

PL-N and PL-C2 are synthetic oligonucleotides (manufactured at the Biomolecular Unit at Lund University (Sweden) in accordance with Applicant's instructions) which were used, with the aid of PCR (Polymerase Chain Reaction) to amplify a clonable gene fragment, called B1-4, which codes for four Ig-binding protein L domains (ala-val-glu-asn-B1-B2-B3-B4-lys-lys-val-asp-glu-lys-pro-glu-glu):

30  
PL-N: 5'-GCTCAGGCGGCGCCCGTAGAAAATAAAGAAGAAACACCAGAAAC-3'  
valqluasnlysqluqluthrproqlu

P1-C2: 5'-CAGCAGCAGCCATGGGTCTCTCTGGTTTTTCGTCAACTTTCTTA-  
35 3'

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Amino acids have been shown under corresponding triplets in the coded strand. DNA-fragments which have been amplified with the aid of PL-N contain the recognition sequence for the restriction enzyme *HpaII* immediately upstream of the triplet which codes for the first amino acid (val) in the expressed protein L-fragment. The fragment that has been cleaved with *HpaII* can be ligated with DNA (in this case, the used expression vector PHD389) which has been cleaved with *NarI*. The construction results in translation of an extra amino acid (ala) immediately upstream of the first amino acid in the protein L-fragment. The DNA-fragment that has been amplified with the aid of PL-C2 will contain the recognition sequence for the restriction enzyme *NcoI* (emphasized) immediately downstream of the sequence which codes for the last amino acid in the expressed protein L-fragment (glu). Amplified fragments which have been cleaved with *NcoI* can be ligated to the *NcoI*-cleaved, PCR-generated protein-asp-CDC-met-fragment (see below).

#### Protein G

It is known that a simple C-domain from protein G will bind to IgG (B. Guss, M. Eliasson, A. Olsson, M. Uhlen, A.-K. Frej, H. Jörnvall, I. Flock and M. Lindberg. 1986. Structure of the IgG-binding regions of streptococcal protein G. *EMBO. J.* 5: 1567-1575). The strength at which a simple C-domain binds to IgG is relatively low ( $5 \times 10^7 \text{ M}^{-1}$ ). A fragment which consists of two C-domains with an intermediate D-region having a length of 15 amino acids, however, has a considerably higher affinity to IgG ( $1 \times 10^9 \text{ M}^{-1}$ ). CDC-N and CDC-C are oligonucleotides which have been used as PCR-primers to amplify a clonable DNA-fragment, designated CDC, which

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codes for two IgG-binding protein G-domains (pro-met-asp-CDC-met).

CDC-N: GGCCATGGACACTTACAAATTAATCCTTAATGGT  
metaspthrtyrlysleuileleuasngly

CDC-C: CAGGTGCACTTATTACATTTTCAGTTACCGTAAAGGTCTTAGT

Amino acids in the resultant sequence have been shown beneath the primer of the coding strand. DNA-fragments which have been amplified with the aid of CDC-N contain the recognition sequence for the restriction enzyme NcoI (marked with a line above the sequence). Cleaved amplified fragments can be ligated with the fragment that has been amplified with the aid of PL-C2 and then cleaved with NcoI. The fragment will therewith be translated to the correct reading frame. DNA-fragments which have been amplified with the aid of CDC-C will contain two so-called stop condons (emphasized) which terminate translation. The recognition sequence for the restriction enzyme SalI (marked with a line above the sequence) follows immediately afterwards, this sequence also being found in the expression vector pHD389 (see Figure 1).

Those sequences which code for the binding properties of protein L (B1-B5) and for protein G (CDC) respectively contain no internal recognition sequences for the restriction enzymes HpaII, SalI or NcoI.

#### Amplification and cloning procedures

PCR (Polymerase Chain Reaction) was carried out in accordance with a protocol described by Saiki et al., 1988; PCR was carried out in a Hybaid Intelligent Heating-block (Teddington, UK): 100 µl of the reaction mixture contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5

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mM  $MgCl_2$ , 100  $\mu g/ml$  gelatine, 300  $\mu M$  with respect to each of the deoxynucleotides (dATP, dCTP, dGTP, dTTP), (Pharmacia). In order to amplify sequences which code for the light-chain binding parts of protein L, there were added 20 pmol of each of the oligonucleotides PL-N and PL-C2, and 10  $\mu l$  of a DNA-solution which contained 0.1 mg/ml of chromosomal DNA from Peptostreptococcus magnus, strain 312. By way of an alternative, 20 pmol were added to each of the oligonucleotide pairs CDC-N and CDC-C and 10  $\mu l$  of a DNA-solution which contained 0.1 mg/ml of chromosomal DNA from a group C streptococcus strain (Streptococcus equisimilis) called C40 (U. Sjöbring, L. Björck and W. Kastern. 1991. Streptococcal protein G: Gene structure and protein binding properties. J. Biol. Chem. 266: 399-405 or with NcoI and SalI (10 U/ $\mu g$  PCR-product), (for CDC) at 37°C. The thus amplified and subsequently cleaved DNA-fragments were then separated by electrophoresis in a 2% (weight by volume) agarose gel (NuSieve agarose, FMC Bioproducts) in a TAE-buffer (40 mM Tris, 20 mM aNa-cetate, 2 mM EDTA, pH 8.0). The resultant fragments, 930 bp (for B1-4) and 390 bp (for CDC) were cut from the gel. The concentration of DNA in the thus separated gel pieces was estimated to be 0.05 mg/ml. The agarose pieces cut from the gel and containing the cleaved, amplified fragments (B1-4 and CDC) were melted in a water bath at 65°C, whereafter they were allowed to cool to 37°C. 10  $\mu l$  (0.5  $\mu g$ ) of this DNA were transferred to a semi-microtube (Sarstedt), preheated to 37°C, whereafter 1  $\mu l$  of the vector pHD389 which had been cleaved with NarI and SalI were added. 1  $\mu l$  10 x ligase buffer (Promega) and 1  $\mu l$  T4 DNA-ligase (1 unit/ $\mu l$ ) were also added. The ligating reaction was permitted to take place at 37°C for 6 hours. The cleaving and ligating conditions recommended by the producer of DNA-ligase and restriction enzymes (Promega) were followed in other respects. The

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ligating reaction was then used to transform E. coli, strain LE392, which had been made competent in accordance with the rubidium-chloride/calcium-dichloride method as described by Kushner (1978). Manipulation of DNA was effected in accordance with molecular biological standard methods (Sambrook et al., 1989).

#### Expression system

The vector PHD389 (see Figure 2) is a modified variant of the plasmid PHD313 (Dalbøge et al., 1989). The vector which was replicated in E. coli (contains origin of replication from plasmid pUC19) is constructed such that DNA-fragments which have been cloned in the cleaving site for NarI will be expressed immediately after, or downstream, of the signal peptide (21 amino acids) from the envelope protein ompA from E. coli. Translation will be initiated from the ATG-codon which codes for the first amino acid (methionine) in the signal peptide. The construction with an E. coli-individual signal sequence which precedes the desired peptide enables the translated peptide to be transported to the periplasmic space in E. coli. This is beneficial since it reduces the risk of degradation of the desired product through the intracellular occurrent enzymes of E. coli. Furthermore, it is easier to purify peptides which have been exported to the periplasmic space. Unique recognition sequences (multiple cloning sequences) for several other restriction enzymes, among them EcoRI, SalI and BamHI are present immediately downstream of the NarI cleaving site. An optimized so-called Shine-Dalgarno sequence (also called ribosomal binding site, RBS) is found seven nucleotides upstream of the ATG-codon in the signal sequence from ompA, this optimized Shine-Dalgarno sequence binding to a complementary sequence in 16S rRNA in the ribosomes and in a manner to decide that the

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translation is initiated in the correct place. The transcription of such DNA as that which is co-transcribed with the signal sequence for ompA is controlled by the  $P_R$ -promotor from coliphage  $\lambda$ . The vector also  
5 contains the gene for cI857 from coliphage  $\lambda$ , the product of which regulates-down transcription from  $P_R$  and the product of which is expressed constitutively. This cI857-mediated down-regulation of transcription from  $P_R$  is heat-sensitive. Transcription which is regulated, or  
10 controlled, from this promotor will be terminated with the aid of a so-called rho-independent transcription terminating sequence which is inserted in the vector immediately downstream of the multiple cloning site. The plasmid also carries the gene for  $\beta$ -lactamase (from the  
15 plasmid pUC19), the product of which permits ampicillin-selection of E. coli clones that have been transformed with the vector.

#### Selection of protein LG-produced clones

20 The transformed bacteria are cultivated on culture plates with LB-medium which also contained ampicillin in a concentration of 100  $\mu$ g/ml. The bacteria were cultivated overnight at 30°C, whereafter they were trans-  
25 ferred to a cultivation cabinet (42°C) and cultured for a further four (4) hours. The plates were stored in a refrigerator overnight. On the following day, the colonies were transferred to nitrocellulose filters. The filters and culture plates were marked, so that the  
30 transferred colonies could later be identified on the culture plate. The culture plates were again incubated overnight at 30°C, so that rests of transferred bacteria colonies remaining on the plates could again grow. The  
35 plates were then stored in a refrigerator. The filter was incubated in 10% SDS for 10 minutes, so as to lyse the bacteria in the colonies on the nitrocellulose

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impression. Filters containing lysed bacteria were then rinsed with a blocking buffer consisting of PBS (pH 7.2) with 0.25% gelatine and 0.25% Tween-20 (four baths of 250 ml at 37°C), whereafter the filter was incubated with radioactively (marked with <sup>125</sup>I according to the chloramine-T-method) marked Ig-κ-chains (20 ng/ml) in PBS with 0.1% gelatine). The incubation process took place at room temperature for four (4) hours, whereafter non-bound radioactively marked protein was rinsed-off with PBS (pH 7.2) containing 0.5 M NaCl, 0.25% gelatine and 0.25% Tween-20 (four baths, 250 ml each at room temperature). All filters were exposed to X-ray film. Positive colonies on the original culture plate were identified. A number of positive colonies were re-cultivated on new plates and new colony-blot experiments were carried out with these plates as a starting material with the intention of identifying E. coli colonies which bind IgG Fc. These tests were carried out in precisely the same manner as that described above with respect to the identification of E. coli-colonies which expressed Ig light-chain-binding protein, with the exception that a radioactively marked (<sup>125</sup>I) IgG Fc (20 ng/ml) was used as a probe. Clones which reacted with both proteins were selected and analyzed with regard to the size of the DNA-fragment introduced in the vector. One of these clones was chosen for production of protein LG, PHDLG. The DNA't taken from this clone and introduced into plasmid pHD389 was sequenced. The DNA-sequence exhibited full agreement with corresponding sequences (B1-B4 and 21 bases in B5) in the gene for protein L from Peptostreptococcus magnus, strain 312, and with C1DC2 sequence in group C streptococcus strain C40. The size and binding properties of the protein produced from clone pHDLG was analyzed with the aid of SDS-PAGE (see Figure 8), dot-blot experiment (see Figure 10) and competitive binding experiments.

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### Production of protein LG

Several colonies from a culture plate with *E. coli* pHDLG were used to inoculate a preculture (LB-medium with an addition of 100 mg/l ampicillin) were cultivated at 28°C overnight. In the morning, the preculture was transferred to a larger volume (100 times the volume of the preculture) of fresh LB-medium containing ampicillin (100 mg/l) and was cultivated in vibrating flasks (200 rpm), (or fermenters) at 28°C. When an absorbance value of 0.5 was reached at 620 nm, the cultivation temperature was raised to 40°C (induction of transcription). The cultivation process was then continued for 4 hours (applies only to cultivation in vibrated flasks). The bacteria were centrifuged down upon termination of the cultivation process. The bacteria were then lysed at 4°C in accordance with an osmotic shock method (Dalbøge et al., 1989). The lysate was adjusted to a pH of 7. Remaining bacteria rests were centrifuged down and the supernatant then purified on IgG-sepharose, in accordance with the protocol earlier described with reference to protein G and protein L. (Sjöbring et al., 1991, Kastern et al., 1992).

The expression system gave about 30 mg/l of protein LG when cultivation in vibrated flasks. A deposition has been made at DSSM, Identification Reference DSSM *E. coli* LE392/pHDLG.

### Example 3

#### Analysis of the binding properties of protein LG

#### Western Blot

Protein G (the C1DC2-fragment), protein L (four B-



domains) and protein LG were isolated with SDS-PAGE (10% acrylamide concentration). The isolated proteins were transferred to nitrocellulose membranes in three similar copies (triplicate). Each of these membranes was incubated with radioactively marked proteins (20 ng/ml: one of the membrane-copies was incubated with human polyclonal IgG, another with human IgG Fc-fragment and the third with isolated human IgG  $\chi$ chains. Non-bound radioactively marked proteins were rinsed off and all filters were then exposed to X-ray film.

#### Slot-blot

Human polyclonal Ig-preparations and Ig-fragments were applied with the aid of a slot-blot appliances on nitrocellulose filters in given quantities (see Figure 10) on three similar copies. Each of these membranes was incubated with radioactively marked proteins (20 ng/ml). One of the membrane copies was incubated with protein LG, another with protein L and the third with protein G. Non-bound radioactively marked proteins were rinsed-off and all filters were then exposed to X-ray film.

The results are shown in Figures 9 and 10.

Other binding experiments have been carried out, with the following results:

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TABLE

Binding of the proteins G, L and LG to immunoglobulins.

Binding protein:	G	K <sub>a</sub>	L	K <sub>a</sub>	LG	K <sub>a</sub>
<u>Immunoglobulin</u>						
<u>Human:</u>						
Polyclonal IgG <sup>*</sup>	+	67 (10)	+	9.0	+	20
IgG subclasses						
IgG <sub>1</sub>	+	2.0	+		+	
IgG <sub>2</sub> <sup>1</sup>	+	3.1	+		+	
IgG <sub>3</sub>	+	6.1	+		+	
IgG <sub>4</sub>	+	4.7	+		+	
IgG fragment						
Fc <sup>*</sup>		+	6.0 (0.5)	-		+
F(ab') <sub>2</sub> <sup>*</sup>	+	0.4 (0.2)	+		+	
kappa	-		+	1.5	+	
lambda	-		(-) <sup>#</sup>			
Other Ig-classes						
IgM	-		+	11.6	+	
IgA	-		+	10.4	+	
IgE	-		+		+	
IgD	-					
<u>Other Species:</u>						
Polyclonal						
Monkey	+		+		+	
Rabbit						
IgG	+	70	+	0.074	+	
IgG-Fc	+	3.0	-		+	
IgG-F(ab') <sub>2</sub>	+	0.44			+	
Mouse	+	41	+	2.6	+	
Rat	+	1.5	+	0.39	+	
Goat	+	14	-		+	

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TABLE (cont'd.)

Binding of the proteins G, L and LG to immunoglobulins.

Binding protein:		G	K <sub>a</sub>	L	K <sub>a</sub>	LG	K <sub>a</sub>
Immunoglobulin							
Bovine	IgG <sub>1</sub>	+	3	-		+	
	IgG <sub>2</sub>	+	2	-		+	
Horse		+		-		+	
Guinea Pig		+		+		+	
Sheep		+		-		+	
Dog		+		-		+	
Pig		+		+		+	
Hamster		+					
Cat		-		-			
Hen		-		-			
Monoclonals <sup>a</sup>							
Mouse							
	IgG <sub>1</sub>	+		+		+	
	IgG <sub>2a</sub>	+		+		+	
	IgG <sub>2b</sub>	+				+	
	IgG <sub>3</sub>	+				+	
	IgM	-		+		+	
	IgA	-		+		+	
Rat							
	IgG <sub>2a</sub>	+		+		+	
	IgG <sub>2b</sub>	+				+	
	IgG <sub>2c</sub>	+				+	

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$K_a$  = affinity constant ( $M^{-1}$ ). \* The numerals within parenthesis disclose the affinity of a recombinant protein G comprised of two IgG-binding domains. # A weak bond to lambda chains exists.

^ Binding to P1 and PLG depends on the type of light chain of Ig.

It will thus be seen that the synthesized hybrid protein LG has a broad binding activity/specificity.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: HighTech Receptro AB
- (B) STREET: c/o Active, Skeppsbron 2
- (C) CITY: MALMO
- (E) COUNTRY: SWEDEN
- (F) POSTAL CODE (ZIP): 211 20
- (G) TELEPHONE: 040/35 07 00
- (H) TELEFAX: 040/ 23 74 05
- (I) TELEX: 32637 Active S

## (ii) TITLE OF INVENTION: Hybridprotein

## (iii) NUMBER OF SEQUENCES: 1

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(EPO)

## (v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: SE PCT/SE93/00375

## (vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: SE 9201331-7
- (B) FILING DATE: 28-APR-1992

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 305 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: protein

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Escherichia coli LE392/pHDL, DSM 7054

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ala Val Glu Asn Lys Glu Glu Thr Pro Glu Thr Pro Glu Thr Asp Ser  
1                   5                   10                   15  
Glu Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Phe Ala Asn Gly Ser  
20                   25                   30  
Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu  
35                   40                   45

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Ala Tyr Ala Tyr Ala Asp Thr Leu Lys Lys Asp Asn Gly Glu Tyr Thr  
 50 55 60  
 Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly  
 65 70 75 80  
 Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala  
 85 90 95  
 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly  
 100 105 110  
 Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu  
 115 120 125  
 Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr  
 130 135 140  
 Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro  
 145 150 155 160  
 Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys  
 165 170 175  
 Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu  
 180 185 190  
 Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr  
 195 200 205  
 Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly  
 210 215 220  
 Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala  
 225 230 235 240  
 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly  
 245 250 255  
 Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu  
 260 265 270  
 Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr  
 275 280 285  
 Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu  
 290 295 300  
 Glu  
 305

## (2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 921 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: unknown

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- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Escherichia coli LE392/pHDL, DSM 7054

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GCGGTAGAAA ATAAAGAAGA AACACCAGAA ACACCAGAAA CTGATTCAGA	50
AGAAGAAGTA ACAATCAAAG CTAACCTAAT CTTTGCAAAT GGAAGCACAC	100
AAACTGCAGA ATTCAAAGGA ACATTTGAAA AAGCAACATC AGAAGCTTAT	150
GCGTATGCAG ATACTTTGAA GAAAGACAAT GGAGAATATA CTGTAGATGT	200
TGCAGATAAA GGTTATACTT TAAATATTAA ATTTGCTGGA AAAGAAAAAA	250
CACCAGAAGA ACCAAAAGAA GAAGTTACTA TTAAAGCAAA CTTAATCTAT	300
GCAGATGGAA AAACACAAAC AGCAGAATTC AAAGGAACAT TTGAAGAAGC	350
AACAGCAGAA GCATACAGAT ATGCAGATGC ATTAAAGAAG GACAATGGAG	400
AATATACAGT AGACGTTGCA GATAAAGGTT ATACTTTAAA TATTAAATTT	450
GCTGGA AAAAG AAAAAACACC AGAAGAACCA AAAGAAGAAG TTACTATTAA	500
AGCAAACCTTA ATCTATGCAG ATGGAAAAAC ACAACAGCA GAATTCAAAG	550
GAACATTTGA AGAAGCAACA GCAGAAGCAT ACAGATATGC TGA CT TATTA	600
GCAAAAGAAA ATGGTAAATA TACAGTAGAC GTTG CAGATA AAGGTTATAC	650
TTTAAATATT AAATTTGCTG GAAAAGAAAA AACACCAGAA GAACCAAAG	700
AAGAAGTTAC TATTAAAGCA AACTTAATCT ATGCAGATGG AAAA ACTCAA	750
ACAGCAGAGT TCAAAGGAAC ATTTGCAGAA GCAACAGCAG AAGCATACAG	800
ATACGCTGAC TTATTAGCAA AAGAAAATGG TAAATATACA GCAGACTTAG	850
AAGATGGTGG ATACACTATT AATATTAGAT TTGCAGGTAA GAAAGTTGAC	900
GAAAAACCAG AAGAATAATA A	921

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 434 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: unknown
  - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein

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(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Escherichia coli* LE392/pHDLG, DSM 7055

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ala	Val	Glu	Asn	Lys	Glu	Glu	Thr	Pro	Glu	Thr	Pro	Glu	Thr	Asp	Ser	1	5	10	15
Glu	Glu	Glu	Val	Thr	Ile	Lys	Ala	Asn	Leu	Ile	Phe	Ala	Asn	Gly	Ser	20	25	30	
Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	Thr	Phe	Glu	Lys	Ala	Thr	Ser	Glu	35	40	45	
Ala	Tyr	Ala	Tyr	Ala	Asp	Thr	Leu	Lys	Lys	Asp	Asn	Gly	Glu	Tyr	Thr	50	55	60	
Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly	65	70	75	80
Lys	Glu	Lys	Thr	Pro	Glu	Glu	Pro	Lys	Glu	Glu	Val	Thr	Ile	Lys	Ala	85	90	95	
Asn	Leu	Ile	Tyr	Ala	Asp	Gly	Lys	Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	100	105	110	
Thr	Phe	Glu	Glu	Ala	Thr	Ala	Glu	Ala	Tyr	Arg	Tyr	Ala	Asp	Ala	Leu	115	120	125	
Lys	Lys	Asp	Asn	Gly	Glu	Tyr	Thr	Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	130	135	140	
Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly	Lys	Glu	Lys	Thr	Pro	Glu	Glu	Pro	145	150	155	160
Lys	Glu	Glu	Val	Thr	Ile	Lys	Ala	Asn	Leu	Ile	Tyr	Ala	Asp	Gly	Lys	165	170	175	
Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	Thr	Phe	Glu	Glu	Ala	Thr	Ala	Glu	180	185	190	
Ala	Tyr	Arg	Tyr	Ala	Asp	Leu	Leu	Ala	Lys	Glu	Asn	Gly	Lys	Tyr	Thr	195	200	205	
Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly	210	215	220	
Lys	Glu	Lys	Thr	Pro	Glu	Glu	Pro	Lys	Glu	Glu	Val	Thr	Ile	Lys	Ala	225	230	235	240
Asn	Leu	Ile	Tyr	Ala	Asp	Gly	Lys	Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	245	250	255	
Thr	Phe	Ala	Glu	Ala	Thr	Ala	Glu	Ala	Tyr	Arg	Tyr	Ala	Asp	Leu	Leu	260	265	270	

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Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr  
 275 280 285

Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu  
 290 295 300

Glu Pro Met Asp Thr Tyr Lys Leu Ile Leu Asn Gly Lys Thr Leu Lys  
 305 310 315 320

Gly Glu Thr Thr Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val  
 325 330 335

Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr  
 340 345 350

Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Glu Val Ile  
 355 360 365

Asp Ala Ser Glu Leu Thr Pro Ala Val Thr Thr Tyr Lys Leu Val Ile  
 370 375 380

Asn Gly Lys Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Ala  
 385 390 395 400

Glu Thr Ala Glu Lys Ala Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val  
 405 410 415

Asp Gly Val Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr  
 420 425 430

Glu Met

## (2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1308 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: DNA (genomic)

## (iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Escherichis coli L392/PHDLG, DSM 7055

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GCGGTAGAAA ATAAAGAAGA AACACCAGAA ACACCAGAAA CTGATTCAGA	50
AGAAGAAGTA ACAATCAAAG CTAACCTAAT CTTTGCAAAT GGAAGCACAC	100
AAACTGCAGA ATTCAAAGGA ACATTTGAAA AAGCAACATC AGAAGCTTAT	150
GCGTATGCAG ATACTTTGAA GAAAGACAAT GGAGAATATA CTGTAGATGT	200

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TGCAGATAAA GGT TATACTT TAAATATTAA ATTTGCTGGA AAAGAAAAAA	250
CACCAGAAGA ACCAAAAGAA GAAGTTACTA TTAAAGCAAA CTTAATCTAT	300
GCAGATGGAA AAACACAAAC AGCAGAATTC AAAGGAACAT TTGAAGAAGC	350
AACAGCAGAA GCATACAGAT ATGCAGATGC ATTAAAGAAG GACAATGGAG	400
AATATACAGT AGACGTTGCA GATAAAGGTT ATACTTTAAA TATTAAATTT	450
GCTGGAAGG AAAAAACACC AGAAGAACCA AAAGAAGAAG TTACTATTAA	500
AGCAAACTTA ATCTATGCAG ATGGAAGAAC ACAAACAGCA GAATTCAAAG	550
GAACATTTGA AGAAGCAACA GCAGAAGCAT ACAGATATGC TGACTTATTA	600
GCAAAAGAAA ATGGTAAATA TACAGTAGAC GTTGCAGATA AAGGTTATAC	650
TTTAAATATT AAATTTGCTG GAAAAGAAAA AACACCAGAA GAACCAAAAG	700
AAGAAGTTAC TATTAAAGCA AACTTAATCT ATGCAGATGG AAAAECTCAA	750
ACAGCAGAGT TCAAAGGAAC ATTTGCAGAA GCAACAGCAG AAGCATACAG	800
ATACGCTGAC TTATTAGCAA AAGAAAATGG TAAATATACA GCAGACTTAG	850
AAGATGGTGG ATACACTATT AATATTAGAT TTGCAGGTAA GAAAGTTGAC	900
GAAAAACCAG AAGAACCCAT GGACACTTAC AAATTAATCC TTAATGGTAA	950
AACATTGAAA GGCGAAACAA CTACTGAAGC TGTGATGCT GCTACTGCAG	1000
AAAAAGTCTT CAAACAATAC GCTAACGACA ACGGTGTTGA CGGTGAATGG	1050
ACTTACGACG ATGCGACTAA GACCTTTACA GTTACTGAAA AACCAGAAGT	1100
GATCGATGCG TCTGAATTAA CACCAGCCGT GACAACTTAC AAAGTTGTTA	1150
TTAATGGTAA AACATTGAAA GGCGAAACAA CTACTAAAGC AGTAGACGCA	1200
GAAACTGCAG AAAAAGCCTT CAAACAATAC GCTAACGACA ACGGTGTTGA	1250
TGGTGTGTTGG ACTTATGATG ATGCGACTAA GACCTTTACG GTAAGTAAA	1300
TGTAATAA	1308

SUBSTITUTE SHEET

Claims

- 1/ Protein L having the ability to bind to the light chains of immunoglobulins, characterized in that the protein L has the following amino acid sequence:

— B1

Ala	Val	Glu	Asn	Lys	Glu	Glu	Thr	Pro	Glu	Thr	Pro	Glu	Thr	Asp	Ser	1	5	10	15
Glu	Glu	Glu	Val	Thr	Ile	Lys	Ala	Asn	Leu	Ile	Phe	Ala	Asn	Gly	Ser	20	25	30	
Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	Thr	Phe	Glu	Lys	Ala	Thr	Ser	Glu	35	40	45	
Ala	Tyr	Ala	Tyr	Ala	Asp	Thr	Leu	Lys	Lys	Asp	Asn	Gly	Glu	Tyr	Thr	50	55	60	
Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly	65	70	75	80
Lys	Glu	Lys	Thr	Pro	Glu	Glu	Pro	Lys	Glu	Glu	Val	Thr	Ile	Lys	Ala	85	90	95	
Asn	Leu	Ile	Tyr	Ala	Asp	Gly	Lys	Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	100	105	110	
Thr	Phe	Glu	Glu	Ala	Thr	Ala	Glu	Ala	Tyr	Arg	Tyr	Ala	Asp	Ala	Leu	115	120	125	
Lys	Lys	Asp	Asn	Gly	Glu	Tyr	Thr	Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	130	135	140	
Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly	Lys	Glu	Lys	Thr	Pro	Glu	Glu	Pro	145	150	155	160
Lys	Glu	Glu	Val	Thr	Ile	Lys	Ala	Asn	Leu	Ile	Tyr	Ala	Asp	Gly	Lys	165	170	175	
Thr	Gln	Thr	Ala	Glu	Phe	Lys	Gly	Thr	Phe	Glu	Glu	Ala	Thr	Ala	Glu	180	185	190	
Ala	Tyr	Arg	Tyr	Ala	Asp	Leu	Leu	Ala	Lys	Glu	Asn	Gly	Lys	Tyr	Thr	195	200	205	
Val	Asp	Val	Ala	Asp	Lys	Gly	Tyr	Thr	Leu	Asn	Ile	Lys	Phe	Ala	Gly	210	215	220	

— B3

AMENDED SHEET

Article 3

54  
Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala  
225 230 235 240  
5 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly  
245 250 255  
Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu  
260 265 270  
10 Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr  
275 280 B5 285  
Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu  
290 295 300  
Glu

15

and variants, subfragments, multiples or mixtures of the  
domains B1-B5 having the same binding properties.

20 2. DNA-sequence, characterized in that  
it codes for the protein according to Claim 1 and has  
the following nucleotide sequence:

25 GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA 18  
GAA GAA GAA GTA ACA ATC AAA GGT AAC GTA ATC TTT GCA AAT GGA AGC 36  
ACA CAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA 54  
GCT TAT GCG TAT GCA GAT ACT TTG AAG AAA GAC AAT GGA GAA TAT ACT 72  
30 GTA GAT GTT CCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 90  
AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 108  
AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA 126  
ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA 144  
35 AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT 162  
ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA 180  
AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA 198

ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA 576  
 5 GCA TAC AGA TAT GGT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA 624  
 GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GGT GGA 572  
 AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 720  
 AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA 768  
 10 ACA TTT GCA GAA CCA ACA GCA GAA GCA TAC AGA TAC GGT GAC TTA TTA 816  
 GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC 864  
 ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA 912  
 15 GAA TAATAA 92

3. A hybrid protein, characterized in  
 that it includes one or more of the B1-B5-domains ac-  
 20 cording to Claim 1 which bind to the light chains in  
 immunoglobulins of all classes, and domains which bind  
 to heavy chains in immunoglobulin G.

4. A hybrid protein according to Claim 3, characterized  
 25 in that the domains which bind to  
 heavy chains in immunoglobulin G are chosen from among  
 the C1- and C2-domains in protein G or from among any  
 other functionally similar proteins which bind to heavy  
 30 chains in immunoglobulin G, and variants, subfragments,  
 multiples or mixtures thereof having the same binding  
 properties.

5. A hybrid protein according to Claim 4, characterized  
 35 in that the hybrid protein has the  
 following amino acid sequence:

AMENDED SHEET

Article 34

Ala Val Glu Asn Lys Glu Glu Thr Pro Glu Thr Pro Glu Thr Asp Ser  
1 10 15  
5 Glu Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Phe Ala Asn Gly Ser  
20 25 30  
Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Lys Ala Thr Ser Glu  
35 40 45  
10 Ala Tyr Ala Tyr Ala Asp Thr Leu Lys Lys Asp Asn Gly Glu Tyr Thr  
50 55 60  
Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly  
65 70 75 80  
Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala  
85 90 95  
15 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly  
100 105 110  
Thr Phe Glu Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Ala Leu  
115 120 125  
20 Lys Lys Asp Asn Gly Glu Tyr Thr Val Asp Val Ala Asp Lys Gly Tyr  
130 135 140  
Thr Leu Asn Ile Lys Phe Ala Gly Lys Glu Lys Thr Pro Glu Glu Pro  
145 150 155 160  
25 Lys Glu Glu Val Thr Ile Lys Ala Asn Leu Ile Tyr Ala Asp Gly Lys  
165 170 175  
Thr Gln Thr Ala Glu Phe Lys Gly Thr Phe Glu Glu Ala Thr Ala Glu  
180 185 190  
Ala Tyr Arg Tyr Ala Asp Leu Leu Ala Lys Glu Asn Gly Lys Tyr Thr  
195 200 205  
30 Val Asp Val Ala Asp Lys Gly Tyr Thr Leu Asn Ile Lys Phe Ala Gly  
210 215 220  
Lys Glu Lys Thr Pro Glu Glu Pro Lys Glu Glu Val Thr Ile Lys Ala  
225 230 235 240  
35 Asn Leu Ile Tyr Ala Asp Gly Lys Thr Gln Thr Ala Glu Phe Lys Gly  
245 250 255

AMENDED SHEET

08325278-102654

Att 21

Thr Phe Ala Glu Ala Thr Ala Glu Ala Tyr Arg Tyr Ala Asp Leu Leu  
260 265 270

5 Ala Lys Glu Asn Gly Lys Tyr Thr Ala Asp Leu Glu Asp Gly Gly Tyr  
275 280 285

Thr Ile Asn Ile Arg Phe Ala Gly Lys Lys Val Asp Glu Lys Pro Glu  
290 295 300

10 Glu Pro Met Asp Thr Tyr Lys Leu Ile Leu Asn Gly Lys Thr Leu Lys  
305 310 315 320

Gly Glu Thr Thr Thr Glu Ala Val Asp Ala Ala Thr Ala Glu Lys Val  
325 330 335

15 Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val Asp Gly Glu Trp Thr Tyr  
340 345 350

Asp Asp Ala Thr Lys Thr Phe Thr Val Thr Glu Lys Pro Glu Val Ile  
355 360 365

20 Asp Ala Ser Glu Leu Thr Pro Ala Val Thr Thr Tyr Lys Leu Val Ile  
370 375 380

Asn Gly Lys Thr Leu Lys Gly Glu Thr Thr Thr Lys Ala Val Asp Ala  
385 390 395 400

Glu Thr Ala Glu Lys Ala Phe Lys Gln Tyr Ala Asn Asp Asn Gly Val  
405 410 415

25 Asp Gly Val Trp Thr Tyr Asp Asp Ala Thr Lys Thr Phe Thr Val Thr  
420 425 430

Glu Met

30 and variants, subfragments, multiples or mixtures of the  
domains B1-B5 having the same binding properties.

6. DNA-sequence, characterized in that  
it codes for a protein according to Claim 5 and has the  
35 following nucleotide sequence:

Article 34

46

5  
10  
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35

GCG GTA GAA AAT AAA GAA GAA ACA CCA GAA ACA CCA GAA ACT GAT TCA 42  
GAA GAA GAA GTA ACA ATC AAA GGT AAC GTA ATC TTT GCA AAT GGA ACC 96  
ACA CAA ACT GCA GAA TTC AAA GGA ACA TTT GAA AAA GCA ACA TCA GAA 144  
GCT TAT GCG TAT GCA GAT ACT TCG AAG AAA GAC AAT GGA GAA TAT ACT 192  
GTA GAT GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GGA 240  
AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 288  
AAC TTA ATC TAT GCA GAT GGA AAA ACA CAA ACA GCA GAA TTC AAA GGA 336  
ACA TTT GAA GAA GCA ACA GCA GAA GCA TAC AGA TAT GCA GAT GCA TTA 384  
AAG AAG GAC AAT GGA GAA TAT ACA GTA GAC GTT GCA GAT AAA GGT TAT 432  
ACT TTA AAT ATT AAA TTT GCT GGA AAA GAA AAA ACA CCA GAA GAA CCA 480  
AAA GAA GAA GTT ACT ATT AAA GCA AAC TTA ATC TAT GCA GAT GGA AAA 528  
ACA CAA ACA GCA GAA TTC AAA GGA ACA TTT GAA GAA GCA ACA GCA GAA 576  
GCA TAC AGA TAT GCT GAC TTA TTA GCA AAA GAA AAT GGT AAA TAT ACA 624  
GTA GAC GTT GCA GAT AAA GGT TAT ACT TTA AAT ATT AAA TTT GCT GCA 672  
AAA GAA AAA ACA CCA GAA GAA CCA AAA GAA GAA GTT ACT ATT AAA GCA 720  
AAC TTA ATC TAT GCA GAT GGA AAA ACT CAA ACA GCA GAG TTC AAA GGA 768  
ACA TTT GCA GAA GCA ACA GCA GAA GCA TAC AGA TAC GCT GAC TTA TTA 816  
GCA AAA GAA AAT GGT AAA TAT ACA GCA GAC TTA GAA GAT GGT GGA TAC 864  
ACT ATT AAT ATT AGA TTT GCA GGT AAG AAA GTT GAC GAA AAA CCA GAA 912  
GAA CCG ATG GAC ACT TAC AAA TTA ATC CTT AAT GGT AAA ACA TTG AAA 960  
GCG GAA ACA ACT ACT GAA GCT GTT GAT GCT GCT ACT GCA GAA AAA GTC 1008  
TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT GAC GGT GAA TGG ACT TAC 1056  
GAC GAT GCG ACT AAG ACC TTT ACA GTT ACT GAA AAA CCA GAA GTG ATC 1104  
GAT GCG TGT GAA TTA ACA CCA GCG GTG ACA ACT TAC AAA CTT GTT ATT 1152  
AAT GGT AAA ACA TTG AAA GCG GAA ACA ACT ACT AAA GCA GTA GAC GCA 1200  
GAA ACT GCA GAA AAA GCG TTC AAA CAA TAC GCT AAC GAC AAC GGT GTT 1248  
GAT GGT GTT TGG ACT TAT GAT GAT GCG ACT AAG ACC TTT ACC GTA ACT 1296  
GAA ATG TAATAA 1308

AMENDED SHEET



- Article
7. DNA-sequence, characterized in that it codes for a protein according to Claims 3, 4 and 5.
  8. A plasmid vector, characterized in that it includes a DNA-sequence according to any one of Claims 2 and 6-8, preferably the vector pHDLG or pHDL according to Fig. 3 or 4.
  9. A host cell, characterized in that it is transformed with the hybrid plasmid according to Claim 9, in particular a host which belongs to the species E. coli, particularly E. coli LE392, or Bacillus subtilis, Saccaromyces cerevisiae, preferably Id. Ref. DSSM E. coli LE392 pHDL and E. coli LE392/pHDLG respectively.
  10. A method for producing a protein according to Claims 1 and 3-5, characterized by cultivating a host cell according to Claim 10 under suitable conditions; accumulating the protein in the culture or lysing the cells and extracting the protein therefrom.
  11. A reagent kit for binding, separating and identifying immunoglobulins, characterized in that it includes a protein according to any one of Claims 1 and 3-5.
  12. A composition, characterized in that it includes a protein according to any one of Claims 1 and 3-5, and optionally additives or carriers.
  13. A pharmaceutical composition, characterized in that it includes a protein according to any one of Claims 1 and 3-5, and optionally a pharmaceutically acceptable carrier or extender.

# ABSTRACT OF THE DISCLOSURE

The invention relates to sequences of protein L which bind to light chains of immunoglobulins. The invention also relates to hybrid proteins thereof which are able to bind to both light and heavy chains of immunoglobulin G, in particular protein LG. The invention also relates to DNA-sequences which code for the proteins, vectors which include such DNA-sequences, host cells which have been transformed with the vectors, methods for producing the proteins, reagent appliances for separation and identification of immunoglobulins, compositions and pharmaceutical compositions and pharmaceutical compositions which contain the proteins.

0332527B 403394

FOR UTILITY/DESIGN  
CIP/PLANT  
ORIGINAL/SUBSTITUTE  
DECLARATIONS

RULE 63 (37 C.F.R. 1.63)  
DECLARATION AND POWER OF ATTORNEY  
FOR PATENT APPLICATION  
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

CUSHMAN  
FORM

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROTEIN L AND HYBRID PROTEINS THEREOF

the specification of which (CHECK applicable BOX(ES))

☐ is attached hereto.

☐ was filed on.

as U.S. Application Serial No.

☐ was filed as PCT international Application No. PCT/ SE93 / 00375 on April 28, 1993

and (if applicable to U.S. or PCT application) was amended on

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above, to the best of my ability I acknowledge the duty to disclose information which is material to the examination of this application in accordance with 37 C.F.R. 1.56(a) and 35 U.S.C. 102 and 103, each as set forth on the reverse side hereof. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing date of this application

PRIOR FOREIGN APPLICATIONS

Number Country

Day/MONTH/Year Filed

PRIORITY CLAIMED

Yes No

9201331-7

28-04-1992

X

I hereby claim the benefit under 35 U.S.C. 120/365 of all United States applications listed below and PCT international applications listed above or below and, insofar as the subject matter of each of the claims of this application is not disclosed in such prior applications in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. 1.56(a) which occurred between the filing date of the prior applications and the national or PCT international filing date of this application

PRIOR U.S. OR PCT APPLICATIONS

Application Serial No

Day/MONTH/Year Filed

Status patented,

pending, abandoned

PCT/SE93/00375

28-04-1993

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the making of such statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

And I hereby appoint Cushman, Darby & Cushman, Eleventh Floor, 1615 L Street, N.W., Washington, D.C. 20036-5601, telephone number 861-3000 (to whom all communications are to be directed) and the below named partners thereof (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Cushman, Darby & Cushman in writing to the contrary.

Paul N. Kokulis 16773  
Allen Kirkpatrick 16749  
George T. Mohler 17753  
James L. Dooley 17710  
Raymond F. Lippitt 17519  
G. Lloyd Knight 17698  
Carl G. Love 18781

Lawrence A. Hymo 19057  
Edgar H. Martin 20534  
William K. West, Jr. 20557  
Kevin E. Joyce 20508  
Edward M. Prince 23429  
Donald B. Deaver 23048  
David W. Brinkman 20817

George M. Sirilla 18221  
William T. Bullinger 25503  
Donald J. Bird 25323  
James R. Longacre 24421  
W. Warren Taltavull 25647  
Michael L. Keller 26751  
Charles R. Donohoe 24546

Watson T. Scott 26581  
Peter W. Gowdey 25872  
Dale S. Lazar 26872  
Glenn J. Perry 28458  
Kendrew H. Colton 30368  
Chris Comuntzis 31097

1) INVENTOR'S SIGNATURE

Inventor's Name (typed)

Lars  
Södra Sandby

First Middle Initial

Björck

Family Name

Date 23 September 1994

Swedish

Citizenship

Residence (City)

(State/Foreign Country)

Sweden

Post Office Address (Include Zip Code)

Kornvägen 40, S-240 17 Södra Sandby

2) INVENTOR'S SIGNATURE

Inventor's Name (typed)

Ulf  
Lund

First

Middle Initial

Sjöbring

Family Name

Date 23 September 1994

Swedish

Citizenship

Residence (City)

(State/Foreign Country)

Sweden

Post Office Address (Include Zip Code)

Lilla Sigridsgatan 1, S-223 50 Lund

3) INVENTOR'S SIGNATURE

Inventor's Name (typed)

First

Middle Initial

Family Name

Date

Citizenship

Residence (City)

(State/Foreign Country)

Post Office Address (Include Zip Code)

4) INVENTOR'S SIGNATURE

Inventor's Name (typed)

First

Middle Initial

Family Name

Date

Citizenship

Residence (City)

(State/Foreign Country)

Post Office Address (Include Zip Code)

FOR ADDITIONAL INVENTORS, check box ☐ and attach sheet with same information and signature and date for each.

Applicant or Patentee: Lars BJÖRCK et al. Attorney's DWB  
 Serial or Patent No.: \_\_\_\_\_ Docket No.: 216764  
 Filed or Issued: October 26, 1994  
 For: PROTEIN L AND HYBRID PROTEINS THEREOF

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
 STATUS (37 CFR 1.9 (f) and 1.27 (b)) — INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9 (c) for purposes of paying reduced fees under section 41 (a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled PROTEIN L AND HYBRID PROTEINS THEREOF described in

☒ the specification filed herewith  
☐ application serial no. \_\_\_\_\_, filed October 26, 1994  
☐ patent no. \_\_\_\_\_, issued \_\_\_\_\_

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9 (c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

☐ no such person, concern, or organization  
☒ persons, concerns or organizations listed below\*

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

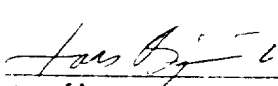
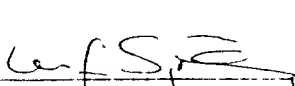
FULL NAME HighTech Receptor AB  
 ADDRESS c/o Active i Malmö AB, Stora Nygatan 61, S-211 37 Malmö  
☐ INDIVIDUAL ☒ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
 ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
 ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Lars Björck	Ulf Sjöbring	
NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
		
Signature of Inventor	Signature of Inventor	Signature of Inventor
<u>September 23 1994</u>	<u>September 23 1994</u>	
Date	Date	Date

55 Rec'd PCT/PTC 26 OCT 1994

08/325278

Applicant or Patentee: Lars BJÖRCK et al. Attorney's DNB  
 Serial or Patent No.: \_\_\_\_\_ Docket No.: M.216764  
 Filed or Issued: October 26, 1994  
 For: PROTEIN L AND HYBRID PROTEINS THEREOF

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
 STATUS (37 CFR 1.9 (f) and 1.27 (c)) — SMALL BUSINESS CONCERN

I hereby declare that I am

- ☐ the owner of the small business concern identified below:  
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN HighTech Receptor AB  
 ADDRESS OF CONCERN c/o Active i Malmö AB, Stora Nygatan 61  
S-211 37 Malmö, Sweden

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.318, and reproduced in 37 CFR 1.9 (d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full time, part time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled PROTEIN L AND HYBRID PROTEINS THEREOF  
 by inventor(s)  
Lars BJÖRCK et al. described in

- ☒ the specification filed herewith  
☐ application serial no. \_\_\_\_\_, filed October 26, 1994  
☐ patent no. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME \_\_\_\_\_  
 ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

NAME \_\_\_\_\_  
 ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

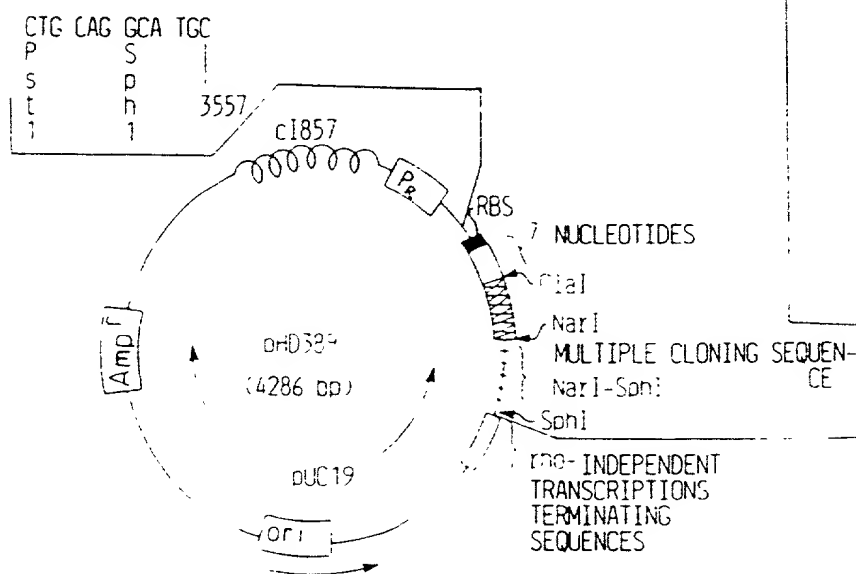
I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING ① JONAS LIND ② KARL-G. BRANZEN  
 TITLE OF PERSON OTHER THAN OWNER CHAIRMAN PROXY MANAGER  
 ADDRESS OF PERSON SIGNING SE "NAME OF COMPANY" SEE "NAME OF CONCERN"

SIGNATURE [Signature] ③ [Signature] DATE 22 SEPT, 1994

3406  
1  
AAGCTTAAGGAGGTTAATCG ATG AAA AAA ACT GCT ATC GCT ATC GCT GTT  
H A C met  
i f RBS l  
n 1 a  
3 2 1  
GCT CTG GCT GGT TTC GCT ACT GTT GCT CAG GCG GCG CCG AGA TCT  
aia<sup>N</sup> B  
a 9  
r 1  
1 2  
AAA CAG GAA TTC GAG CTC GGT ACC CCG GGA TCC TCT AGA GTC GAC  
E S K X B X S  
C a p m a b a  
O C h a m a l  
I 1 1 1 1 1 1



██████ SIGNAL PEPTIDE FOR THE  
SEQUENCE FROM ompA

■  
RBS = RIBOSOMAL AGGAGG  
BINDING SEQUENCE  
P<sub>R</sub> = "RIGHT" PROMOTOR FROM COLIPHAGE λ

c1857 THE GENE FOR A HEAT-SENSITIVE REPRES-  
SOR-PROTEIN FROM COLIPHAGE λ

FIG. 1 PLASMA pHD 389. THE RIBOSOMAL  
BINDING-SEQUENCE (EMPHASIZED WITH  
A FULL LINE), THE SEQUENCE FOR SIGNAL PEPTIDE FROM ompA  
(FROM E.coli) (DOTTED LINE) AND RECOGNITION SEQUENCE FOR  
SEVERAL RESTRICTION ENZYMES ARE SHOWN.

#### MULTIPLE CLONING SEQUENCE

NarI  
BglII  
EcoRI  
SacI  
KpnI  
XmaI  
BamHI  
XbaI  
SalI  
PstI  
SphI

UNIQUE  
CLEAVING  
SITES

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PROTEIN LG

GCGTAGAAAAATAAAGAAGAAACACCCAGAAACACCCAGAAACTGATTCAGAAGAAGAGTA  
 AlaValGluAsnLysGluGluThrProGluThrProGluThrAspSerGluGluGluVal  
 60 20  
 ACAATCAAAGCTAACCTAATCTTTGCAAATGGAAGCACACAACTGCAGAAATTCAAAGGA  
 ThrIleLysAlaAsnLeuIlePheAlaAsnGlySerThrGlnThrAlaGluPheLysGly  
 120 40  
 ACATTGAAAAAGCAACATCAGAAAGCTTATGCGTATGCAGATACTTTGAAGAAAGACAAT  
 ThrPheGluLysAlaThrSerGluAlaTyrAlaTyrAlaAspThrLeuLysLysAspAsn  
 180 60  
 GGAGAAATATACTGTAGATGTTGCAGATAAAAGGTTATACTTTAAATATTAAATTTGCTGGA  
 GlyGluTyrThrValaspValAlaaspLysGlyTyrThrLeuAsnIleLysPheAlaGly  
 240 80  
 AAAGAAAAACACCCAGAAACCAAAAGAAAGAAAGTACTATTAAGCAAACTTAATCTAT  
 LysGluLysThrProGluGluProLysGluGluValThrIleLysAlaAsnLeuIleTyr  
 300 100  
 GCAGATGGAAAAACACAAACAGCAGAAATTCAAAGGAACATTTGAAGAAAGCAACAGCAGAA  
 AlaAspGlyLysThrGlnThrAlaGluPheLysGlyThrPheGluGluAlaThrAlaGlu  
 360 120

FIG.2  
(CONT.)

[illegible]FIG. 2  
(CONT.)

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GCAACAGCAGAAGCATACAGATACGCTGACTTATTAGCAAAGAAATGGTAAATATAACA  
AlaThrAlaGluAlaTyArgTyAlaAspLeuLeuAlaLysGluAsnGlyLysTyThr

GCAGACTTAGAAGATGGTGATACACTATTAAATATTAGATTTCAGGTTAAGAAAAGTTGAC  
AlaAspLeuGluAspGlyGlyTyThrIleAsnIleArgPheAlaGlyLysLysValasp

GAAAAACCAGAAAGAACCCCATGGACACTTACAAATTAATCCTTAATGGTAAACATTGAAA  
GluLysProGluGluProMetAspThrTyLysLeuIleLeuAsnGlyLysThrLeuLys

GGCGAAACAACTACTGAAGCTGTGTGATGCTGCTACTGCAGAAAAAGTCTTCAAAACAATAC  
GlyGluThrThrThrGluAlaValaspAlaAlaThrAlaGluLysValPheLysGlnTy

GCTAACGACAACGGTGTGACCGGTGAATGGACTTACGACGATGCGACTAAGACCTTTACA  
AlaAsnAspAsnGlyValaspGlyGluTrpThrTyAspAspAlaThrLysThrPheThr

GTTACTGAATAAACCCAGAAAGTGCATGCGTCTGAAATTAACACCAGCCGTGACAACTTAC  
ValThrGluLysProGluValIleaspAlaSerGluLeuThrProAlaValThrThrTy

FIG. 2  
(CONT.)

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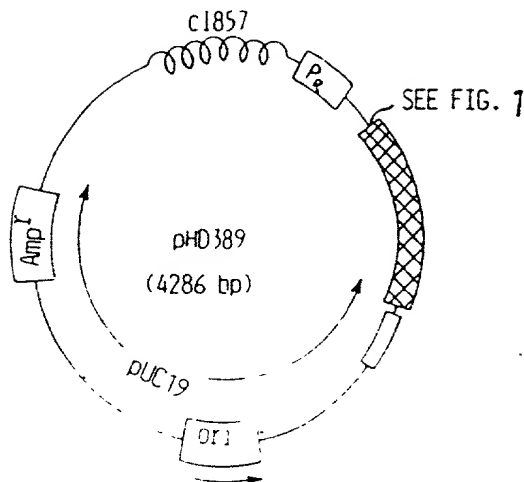
V	V	V	V	V	V	1200
AAACTTGTATTATTAATGGTAAACATTTGAAAGCGGAAACAACACTACTAAAGCAGTAGACGCA						
LysLeuValIleAsnGlyLysThrLeuLysGlyGluThrThrThrLysAlaValAspAla						400
V	V	V	V	V	V	1260
GAAACTGCAGAAAAAGCCTTCAAAACAATACGCTAACGACAAACGGTGTGATGGTGTGG						
GluThrAlaGluLysAlaPheLysGlnTyrAlaAsnAspAsnGlyValAspGlyValTrp						420
V	V	V	V	V	V	1308
ACTTATGATGATGCGACTAAGACCTTTACGGTAACTGAAATGTAATAA						
ThrTyrAspAspAlaThrLysThrPheThrValThrGluMet						434

FIG. 2

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FIG. 3 SCHEMATIC OVERALL VIEW OF THE PRODUCTION OF PROTEIN L



(A) CLEAVE WITH  
NarI/BamHI

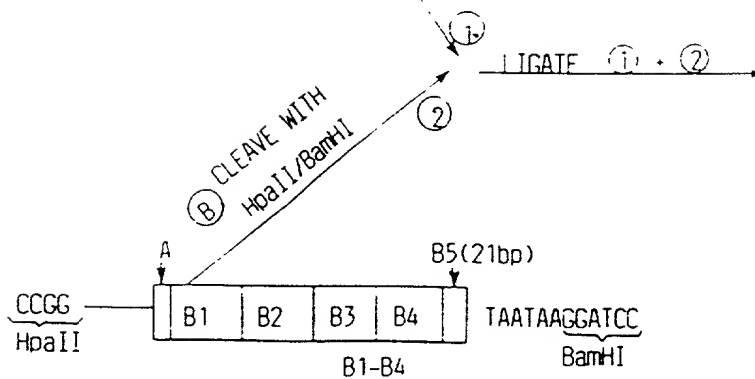


FIG. 3(1)

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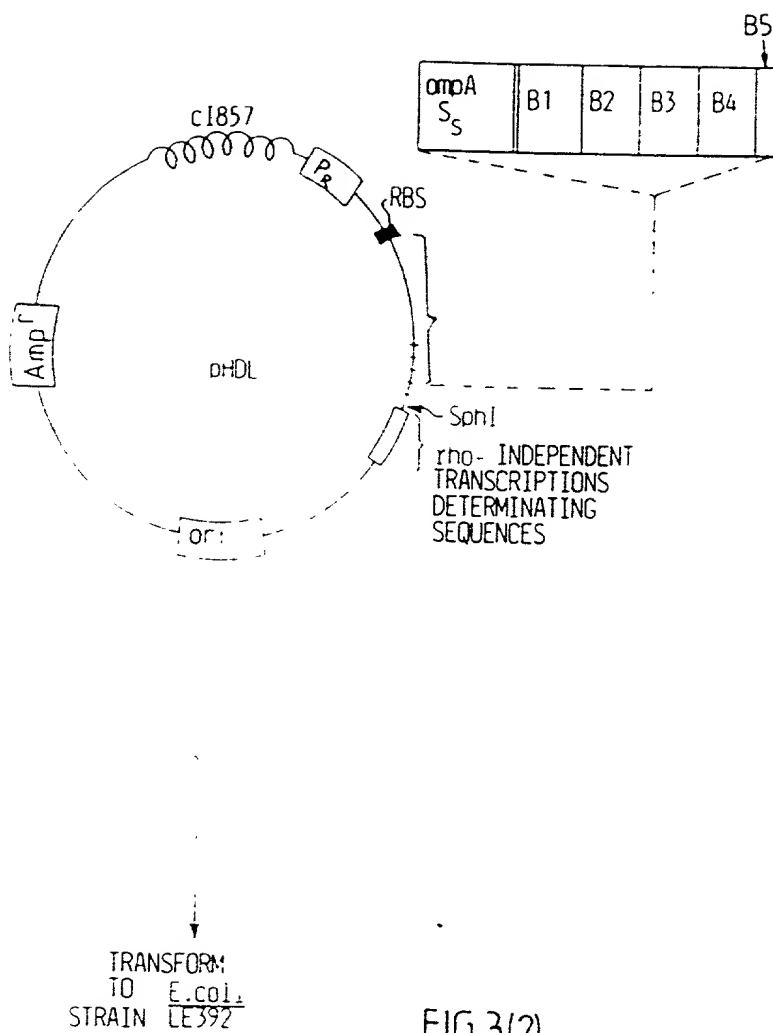


FIG.3(2)

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FIG. 4

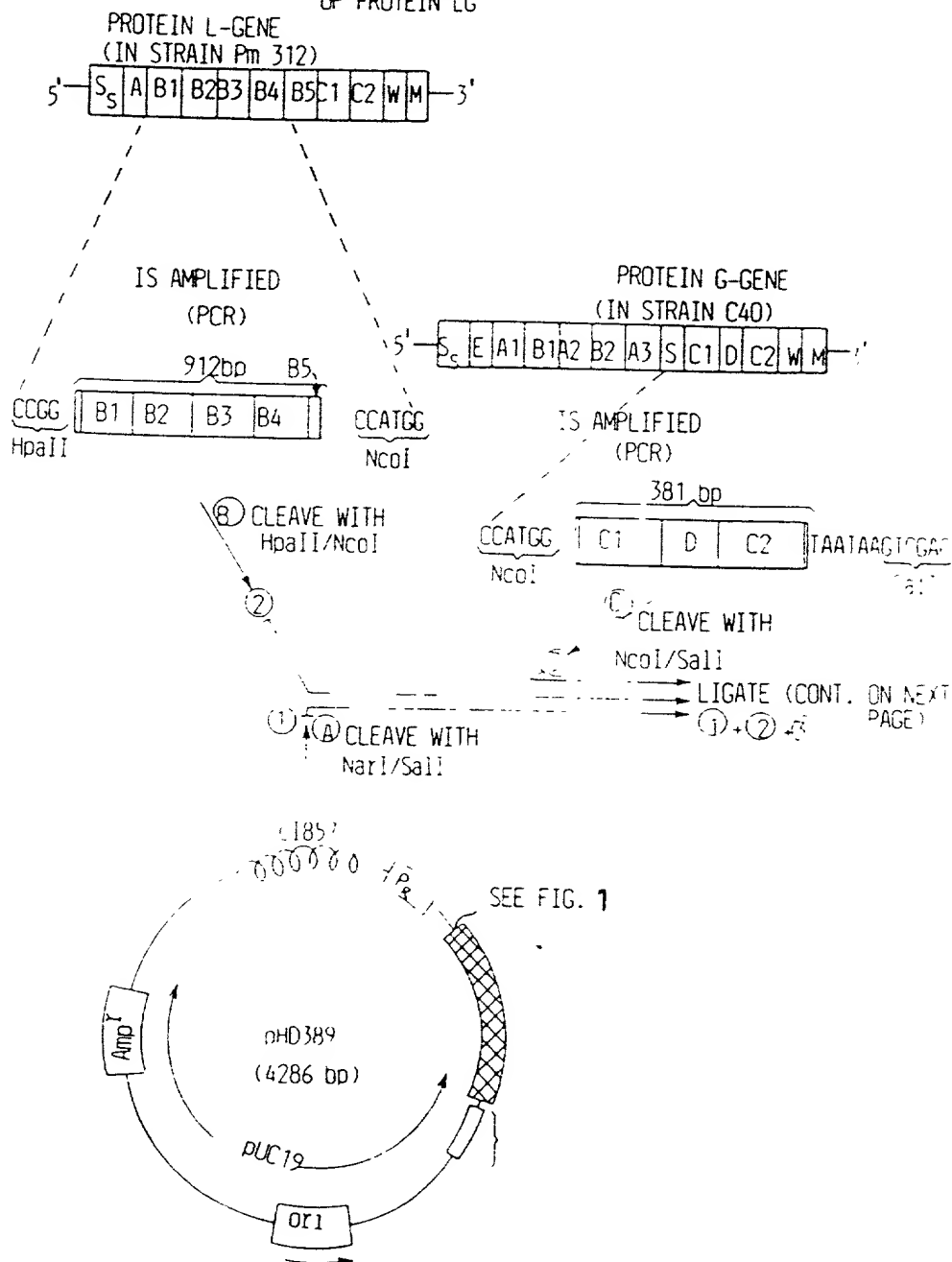
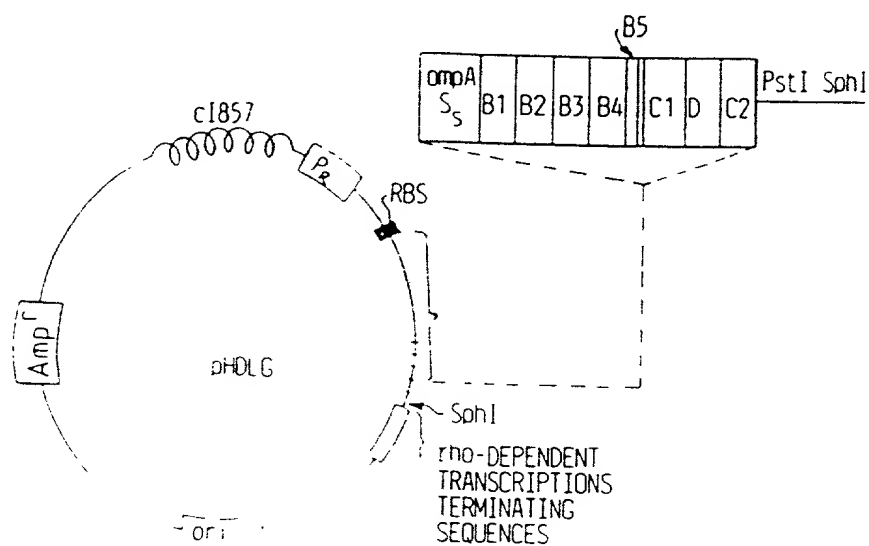
SCHEMATIC OVERALL VIEW OF PRODUCTION  
OF PROTEIN LG

FIG.4(1)

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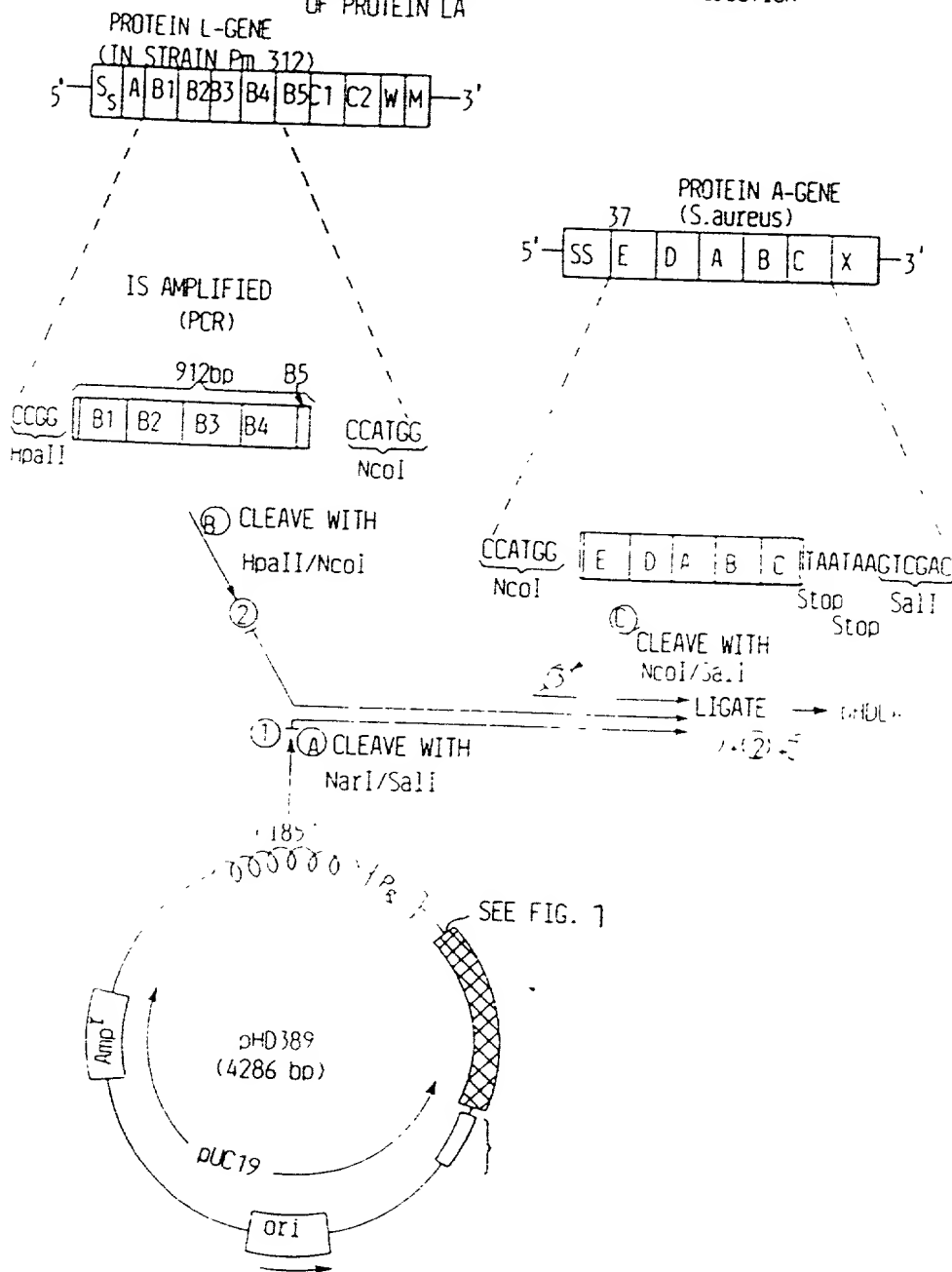
TRANSFORM  
TO E. coli  
STRAIN LE392

FIG.4(2)

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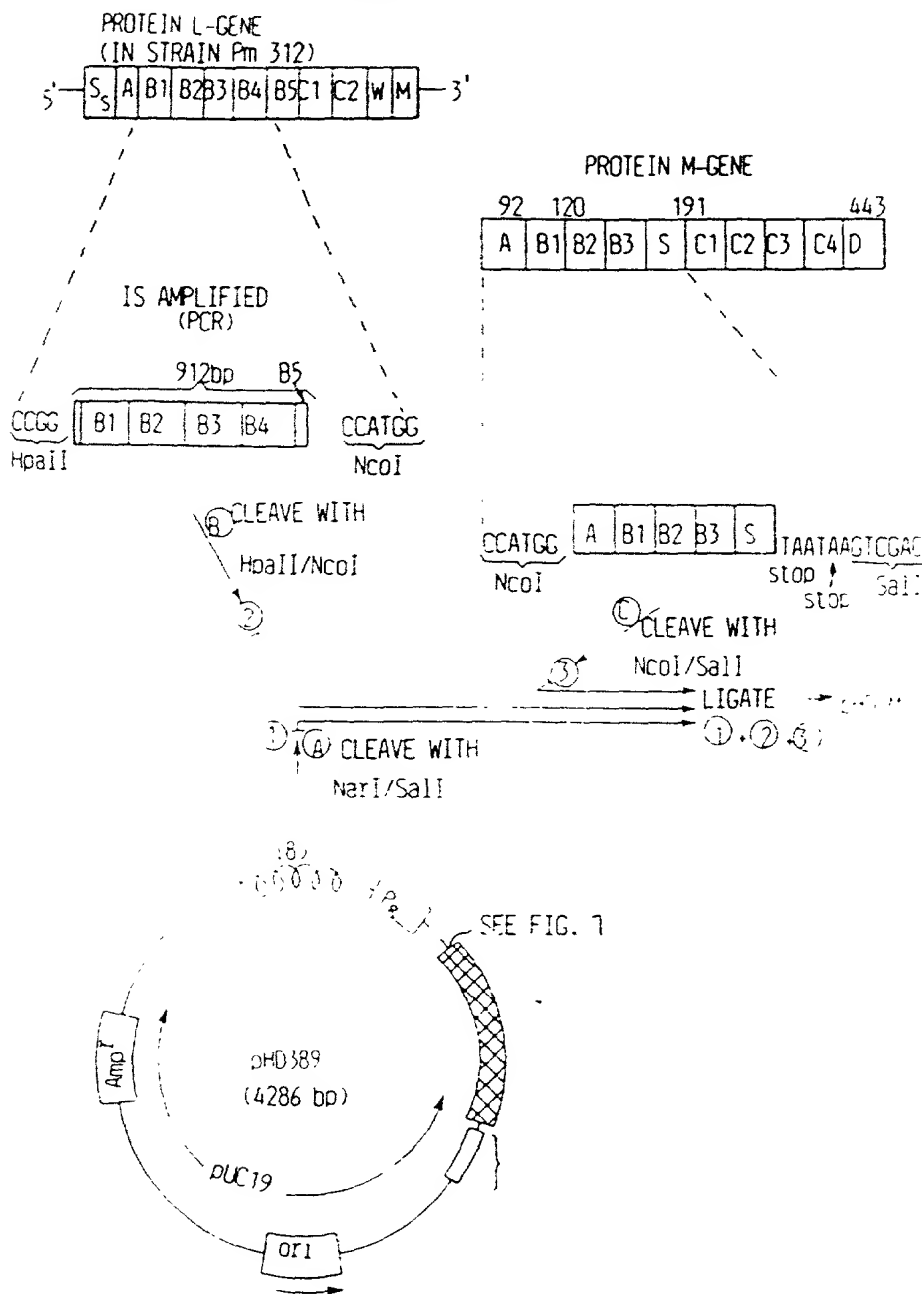
FIG. 5a

SCHEMATIC OVERALL VIEW OF THE PRODUCTION  
OF PROTEIN LA

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FIG.5b SCHEMATIC OVERALL VIEW OF THE PRODUCTION OF PROTEIN LM

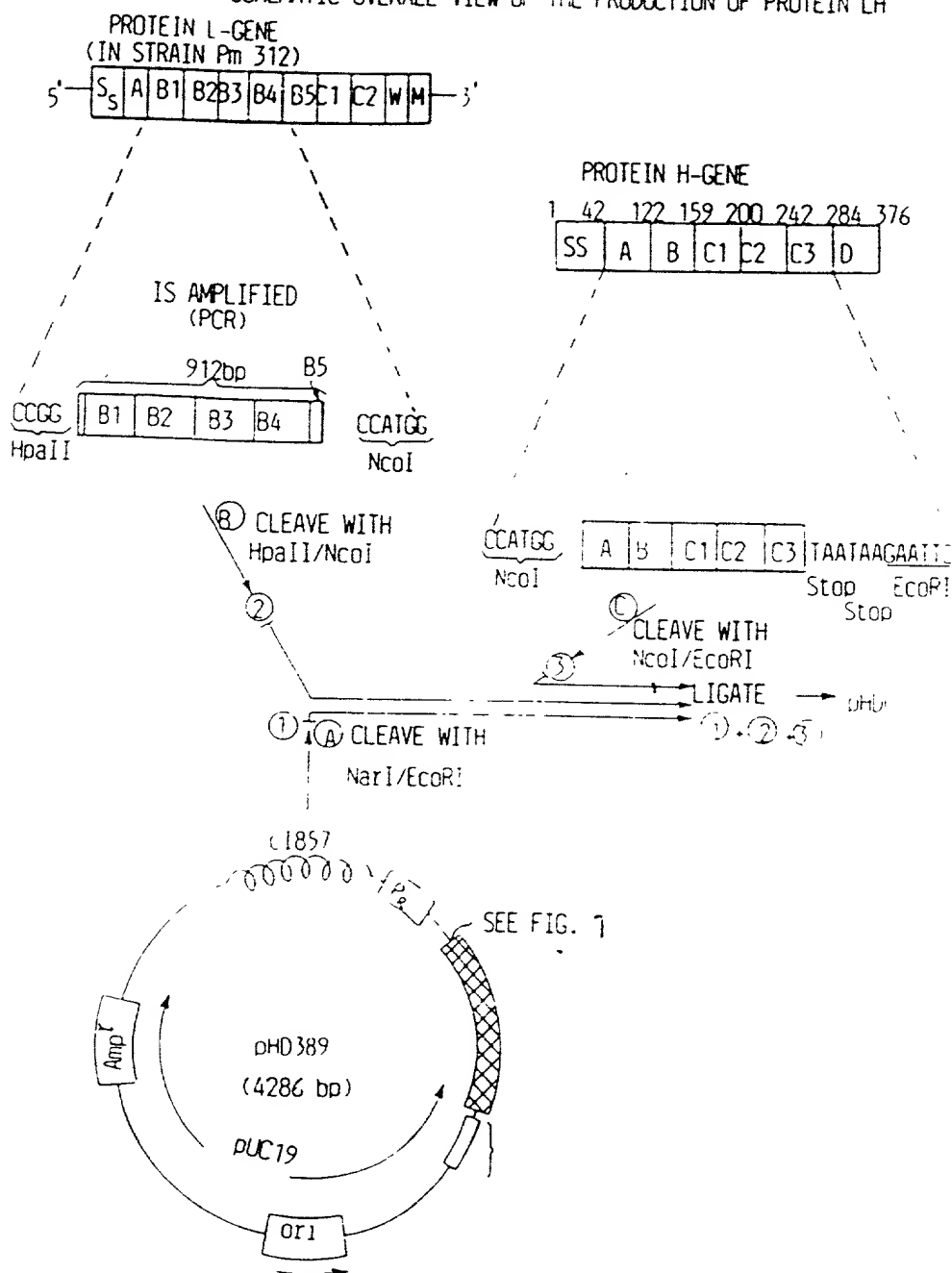


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FIG. 5c SCHEMATIC OVERALL VIEW OF THE PRODUCTION OF PROTEIN LH



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AACGGTGGTAACTCCTAGGGAAAGTTATAGAGATCTTGCAGCAACAAATCCCGCAATA<sup>Y</sup>  
 AsnGlyAspGlyAsnProArgGluValIleGluAspLeuAlaIleAsnAsnProAlaIle<sup>60</sup>  
 20  
 CAAATATACGTTTACGTCACGAAACAAAGGACTTAAAGCGGAGATTAGAGAAATGCAATG<sup>Y</sup>  
 GlnAsnIleArgLeuArgHisGluAsnLysAspLeuLysAlaArgLeuGluAsnAlaMet<sup>120</sup>  
 40  
 GAAGTTGCAGGAAGAGATTTTAAGAGAGAGCTGAAGAACTTGAAAAAGCAAAACAAGCCCTTA<sup>Y</sup>  
 GluValAlaGlyArgAspPheLysArgAlaGluGluLeuGluLysAlaLysGlnAlaLeu<sup>180</sup>  
 60  
 GAGACCAGCGTAAAGATTTAGAACTAAATTAAGAGAACTACAACAAGACTATGACTTA<sup>Y</sup>  
 GluAspGlnArgLysAspLeuGluThrLysLeuLysGluLeuGlnGlnAspTyrAspLeu<sup>240</sup>  
 80  
 GCAAAAGGAATCAACAAGTTGGGATAGACAAAGACTTGAAAAAGAGTTAGAAAGAGAAAAAG<sup>Y</sup>  
 AlaLysGluSerThrSerTrpAspArgGlnArgLeuGluLysGluLeuGluLysLys<sup>300</sup>  
 100  
 GAGCTCTTGAAATTAGCGATAGACCAGGCAAGTCGGGACTACCATAGAGCTACCGCTTTA<sup>Y</sup>  
 GluAlaLeuGluLeuAlaIleAspGlnAlaSerArgAspTyrHisArgAlaThrAlaLeu<sup>360</sup>  
 120  
 GAAAAAGAGTTAGAAAGAGAAAAAGAAAGGCTCTTGAAATTAGCGATAGACCAGGAGTCAG<sup>Y</sup>  
 GluLysGluLeuGluGluLysLysAlaLeuGluLeuAlaIleAspGlnAlaSerGln<sup>420</sup>  
 140  
 GACTATAATAGAGCTAACGTCCTTAGAAAAAGAGTTAGAAACGATTACTAGAGAACAGAG<sup>Y</sup>  
 AspTyrAsnArgAlaAsnValLeuGluLysGluLeuGluThrIleThrArgGluGlnGlu<sup>480</sup>  
 160  
 ATTAATCGTAATCTTTTAGGCAATGCAAAACTTGAACCTTGATCAACTTTCATCTGAAAAA<sup>Y</sup>  
 IleAsnArgAsnLeuLeuGluGluLysAlaLysLeuGluLeuAspGlnLeuSerSerGluLys<sup>540</sup>  
 180

FIG. 7 (CONT.)

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GAGCAGCTAACGATCGAAAGAGCAAACTT<sup>Y</sup>BAGGAAGAAACAAATCTCAGACGCAAGT<sup>Y</sup>  
 GluGlnLeuThrIleGluLysAlaLysLeu<sup>190</sup>GluGluLysGlnIleSerAspAlaSer<sup>Y</sup> 600  
 200  
 CGTCAAAGCC<sup>Y</sup>TCGTGACTTGGACGCATCACGTGAAGCTAAGAAACAGGTTGAAGAA<sup>Y</sup>  
 ArgGlnSerLeuArgArgAspLeuAspAlaSerArgGluAlaLysLysGlnValGluLys<sup>Y</sup> 660  
 220  
 GATTAGCAAAC<sup>Y</sup>TGACTGCTGAAC<sup>Y</sup>TGATAAGGTTAAAGAAAGACCAACAAATCTCAGAC<sup>Y</sup>  
 AspLeuAlaAsnLeuThrAlaGluLeuAspLysValLysGluAspLysGlnIleSerAsp<sup>Y</sup> 720  
 240  
 GCAAGCCGTC<sup>Y</sup>CAACGGCTTCGCCGTGACTTGGACGCATCACGTGAAGCTAAGAAACAGGTT<sup>Y</sup>  
 AlaSerArgGlnArgLeuArgArgAspLeuAspAlaSerArgGluAlaLysLys<sup>Y</sup> 780

FIG.7 (CONT.)

Amino acid sequence and nucleic acid sequence for protein M1, IgG-binding somewhere between amino acid 1-190.

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[illegible]

GAAAAGATTAGCAACTTGACTGCTGAAC TTGATAAGGTAAAGAAAGAAAACAAATC GluLysAspLeuAlaAsnLeuThrAlaGluLeuAspLysValLysGluLysGlnIle	840 890
TCAGACGCAAGCCGTCACGGGCTTCGCCGTGACTTGGACGCATCACGTGAAGCTAAGAAA SerAspAlaSerArgGlnArgLeuArgArgAspLeuAspAlaSerArgGluAlaLysLys	900 930
CAAGTTGAAAAGCTTTAGAAGAGCAACAGCAAAATTAGCTGCTCTTTGAAAAAACTTAAC GlnValGluLysAlaLeuGluLysAlaAsnSerLysLeuAlaAlaLeuGluLysLeuAsn	960 990
AAAGAGCTTGAAGAAAGCAAGAAATTAAACAGAAAAAGAAAGCTGAAC TACAAGCAAAA LysGluLeuGluGluSerLysLysLeuThrGluLysGluLysAlaGluLeuGlnAlaLys	1020 1050
CTTGAGCAGAAAGCAAAAGCAGCTCAAGAAACAAATTAGCGAAACAAAGCTGAAGAACTCGCA LeuGluAlaGluAlaLysAlaLeuLysGluGlnLeuAlaLysGlnAlaGluLysAla	1080 1110
AAACTAAGAGCTGGAAAAGCATCAGACTCACAAACCCCTGATACAAAACAGGAAACAAA LysLeuArgAlaGluLysAlaSerAspSerGlnThrProAspThrLysProGlyAsnLys	1140 1170
GCTGTTCCAGGTAAAGGTCAGGCACCACAGCAGGTACAAAACCTAACC AAAACAAAGCA AlaValProGlyLysGlyGlnAlaProGlnAlaGlyThrLysProAsnGlnAsnLysAla	1200 1230
CCAATGAAGGAAACTAAGAGACAGATTACCATCAACAGGTGAACAGCTAACCCATTCTTC ProMetLysGluThrLysArgGlnLeuProSerThrGlyGluThrAlaAsnProPhe	1260 1290
ACAGCGGCACGGCTTACTGTTATGGCAACAGCTGGAGTAGCAGCAGTTGTAAAACGCAAA ThrAlaAlaArgValThrValMetAlaThrAlaGluValAlaAlaValValLysArgLys	1320 1350
GAAGAAAACCTAA GluGluAsn	1329 1343

FIG. 7

FIG. 7

GAAGAAACTAA<sup>y</sup>  
GluGluasn, > 1329  
443

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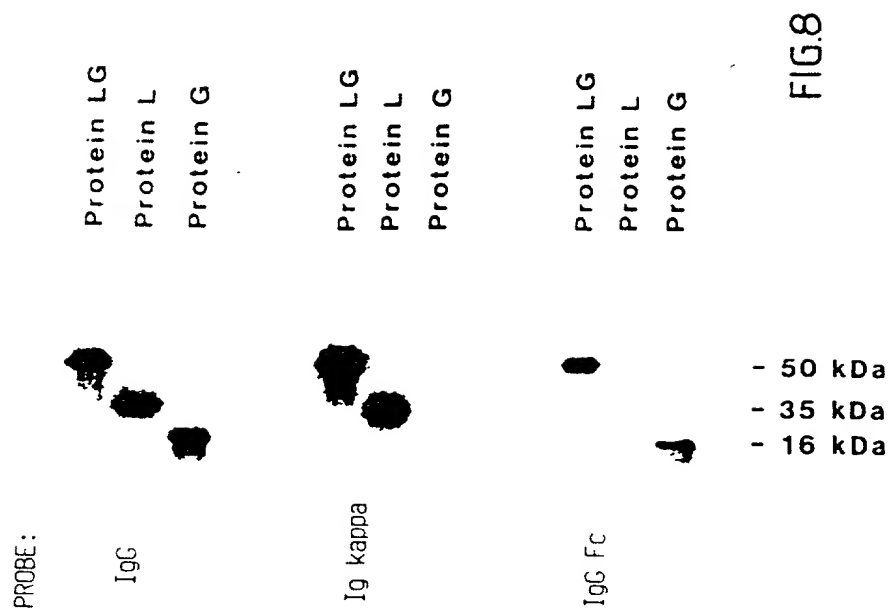


FIG. 8

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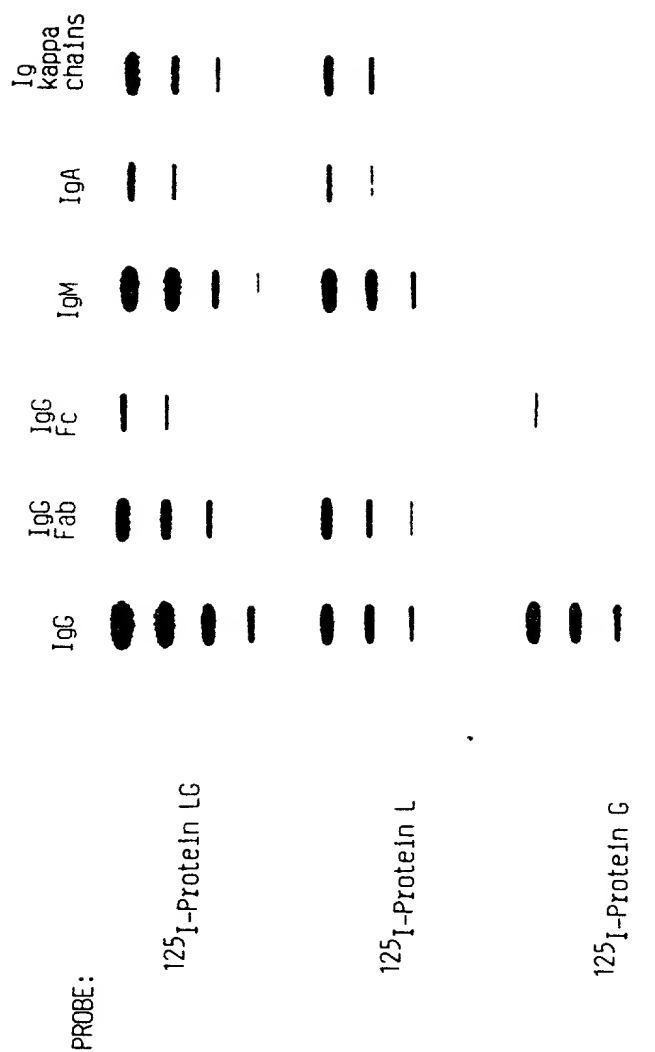



FIG. 9

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SERIAL NUMBER 08/325,278		FILING DATE 10/26/94	CLASS 530	GROUP ART UNIT 1806	
APPLICANT	LARS BJORCK, SODRA SANDBY, SWEDEN; ULF SJOBRING, LUND, SWEDEN.				
	**CONTINUING DATA***** VERIFIED      THIS APPLN IS A 371 OF      PCT/SE93/00375      04/28/93				
	**FOREIGN/PCT APPLICATIONS***** VERIFIED      SWEDEN      9201331-7      04/28/92				
***** SMALL ENTITY *****					
STATE OR COUNTRY SEX	SHEETS DRAWING 18	TOTAL CLAIMS 13	INDEPENDENT CLAIMS 1	FILING FEE RECEIVED \$490.00	ATTORNEY DOCKET NO. 216764
ADDRESS	CUSHMAN DARBY & CUSHMAN ELEVENTH FLOOR 1615 L STREET NW WASHINGTON DC 20036-5601				
TITLE	PROTEIN L AND HYBRID PROTEINS THEREOF				
This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application which is identified above.  By authority of the COMMISSIONER OF PATENTS AND TRADEMARKS					
Date		Certifying Officer			



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